STUDIES ON DISORDERS OF GROWTH IN CHILDHOOD

WITH PARTICULAR REFERENCE TO THE

ANTERIOR PITUITARY GLAND.

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for Consideration for the Degree

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by

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STUDIES ON DISORDERS OF GROWTH IN CHILDHOOD WITH PARTICULAR REFERENCE TO THE ANTERIOR PITUITARY GLAND.

SUMMARY

Forty-one boys and twenty-nine girls were studied and sixty-seven of these children were investigated because of their small size. Attention was paid to the following points in the history and clinical examination; chief complaint, birth weight, birth order, pregnancy and confinement, maternal age at birth, parental heights, height and skeletal age, and the age at which "small size" was noticed.

Tests of anterior pituitary function were applied and these were based upon the response of target organs. Eighteen children were found to have a primary disease process initially unrelated to the anterior pituitary, and certain of these were discussed, e.g. chronic regional ileitis, and erythrogenesis imperfecta with transfusion haemosiderosis. The association of anaemia with hypopituitarism in two girls was considered, and twelve children with a history of convulsions were examined in detail.

Thirty-seven children were investigated using all the tests of endocrine function available in this study. They showed no clinical evidence of a destructive pituitary lesion and they were

grouped according to their biochemical findings. No conclusive evidence was obtained on comparison of these groups with certain physical attributes, e.g. birth weight and parental height.

A classification of endocrine disorders of growth in pre-pubertal children based upon biochemical defects was proposed for research purposes.

Metabolic studies with Human Growth Hormone were carried out in four children and they showed variable responses but no evidence of hypercalciuria which has been described by others.

Attempts to establish Read's method of assay of Human Growth Hormone were unsuccessful.

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INTRODUCTION

Those individuals who fall markedly below the average height of a species may be termed "dwarfs". There are ethnic dwarfs as well as dwarfed individuals in normal races, and there is frequent reference to dwarf growth in the legends and literature of most languages. The early reports have been exhaustively, if uncritically, reviewed by Rischbieth (1912).

Most of the causes of dwarfism in children are well-known and are listed in the standard texts (e.g. Wilkins, 1957). They include primary skeletal abnormalities, nutritional disorders and renal disease. Many of these causes may be excluded on clinical examination alone, but there is a large group of children which defies accurate diagnosis and this includes the so-called genetic and endocrine dwarfs.

Kundrat (1891) was the first to show that many varied pathological processes could produce shortness of stature. Previously, investigation was confined to measurement and detailed clinical description, which caused the literature to be overburdened with eponyms and which created chaos in classification. It was Hutchison (1900) and Benda (1900) who first suggested the possible role of the pituitary gland in dwarfism, and in one of three cases reported by Levi (1908) there were definite clinical signs of a pituitary lesion. Since Erdheim's description of a dwarf with a pituitary tumour (1916), there have been several recorded instances of dwarfs with proven hypophyseal lesions

(e.g. Falta, 1927), but in most cases the aetiology remains unknown.

The scientific investigation of pituitary function was not begun until the late 19th century when attempts were made to study the effects of ablation in animals. Reports of such experiments were conflicting until Smith (1916) and Allen (1916), working independently, showed that hypophysectomy in the early frog embryo resulted in thyroid atrophy, a failure of metamorphosis, a slow rate of growth, and a reduction in the number of melanophores and pigment granules. The subsequent discovery by Evans and Long (1922) of the growth-enhancing effect of an anterior pituitary extract in the rat, along with Smith's description (1926) of the parapharyngeal approach for hypophysectomy formed the basis for the future understanding of pituitary physiology.

The early indications that the pituitary was concerned with somatic growth in mammals (e.g. Crowe, Cushing and Homans, 1910; Aschner, 1912) were confirmed in 1930 when Smith reported that the most profound effects of hypophysectomy in the rat were dwarfism, atrophy of the thyroid, adrenal cortex and gonads, loss of libido, and the failure of liver, spleen and kidneys to achieve a normal weight. Smith also noticed the persistence of infantile characteristics in the young hypophysectomised animal and he described how the daily administration of fresh pituitary extracts caused complete reversal of his findings.

Such fundamental experiments stimulated research in biochemistry and in its allied disciplines. During the past twenty years with new and refined methods of protein fractionation, there have been many reports on the isolation and characterisation of the active principles of the anterior pituitary. Thus Li, Evans and Simpson (1943) and Sayers, White and Long (1943) extracted adrenocorticotrophic hormone from sheep and ox pituitaries, and this led to the isolation of a melanocyte-stimulating factor by Lerner, Shizume and Bunding (1954). Further studies on the specific growth-promoting hormone resulted in the isolation of highly purified crystalline preparations by Li, Evans and Simpson (1945) and Wilhelmi, Fishman and Russell (1948). At present seven anterior pituitary hormones are available in pure form or as compounds with high specific activity; these are adrenocorticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), growth hormone (GH), luteinising hormone (LH), melanocyte-stimulating hormone (MSH), prolactin (P), and thyroid-stimulating hormone (TSH).

The recognition of these compounds has led to the development of methods of assay but as yet none of these is applicable to routine clinical use. Anterior pituitary function must therefore be assessed by the measurement of the responses of the target organs. It is not uncommon to find instances of dwarfism which have been ascribed to endocrine causes on the most equivocal

observation. Rational therapy cannot be based upon assumption, therefore the work of this thesis was undertaken to investigate disorders of growth in children with particular reference to the anterior pituitary gland.

The studies to be described form three sections. The first is concerned with the evaluation of the endocrine status of dwarfed children using tests of end-organ function, the second comprises metabolic studies with human growth hormone, and the third deals with methods of assay of human growth hormone in serum or plasma.

SECTION 1

EVALUATION OF THE ENDOCRINE STATUS OF CHILDREN OF SHORT STATURE

Twenty-nine girls and forty-one boys were studied during the author's tenure of the post of Registrar to the Department of Chemical Pathology, The Hospital for Sick Children, Great Ormond Street. Sixty-seven of these children were investigated on account of their small size even where the initial complaint was apparently unrelated. Two boys, in whom a diagnosis of suprasellar neoplasm had been made, were included in this series, despite the fact that one was about the 45th percentile for height (against chronological age). A third boy, who was not dwarfed, was also considered. It was suspected that he had a pituitary lesion.

The approach to the study of disorders of growth in this instance was essentially biochemical, but particular attention was paid to the following points in the history and physical examination:-

Chief complaint.
Birth weight.
Birth order.
Pregnancy and confinement.
Maternal age at birth.
Parental heights.
Height and weight of patient.
Age at which disturbance of growth was noted.

It was not possible to obtain adequate data in all of the

children because three were adopted, two were private patients, and in others there was no opportunity to take or to confirm a history.

The tests of anterior pituitary function were based upon measurements of the response of the target organs. These investigations were considered the best at our disposal:-

The insulin tolerance test.
The response of the adrenal gland to exogenous and endogenous ACTH.
Plasma electrolytes and blood urea before and after ACTH.
Serum protein-bound iodine.
Basal metabolic rate.
Thyroid uptake of ^{132}I.
Urinary gonadotrophins.

Again, it was not possible to apply all of these tests in each child.

The values obtained from the history on maternal admission are approximate; for example, it was not possible to verify details of each pregnancy and confinement from medical records and the results must serve only as a guide for further enquiry. It was not possible, again, to make accurate measurements of parental heights.

The age at which "small size" was noted is also open to subjective variation and the results must be considered with reservations.

The heights and weights of the children studied were measured in the Growth Clinic (Dr. J.M.Tanner), or on the ward in the case of those children who were not referred to that clinic.

Data on the history and physical examination are given in the following tables (Tables 1 to 12, inclusive).

Table 1.

BOYS

Chief complaint	Number of patients
Small size.	19
Small size and convulsions.	3
Small size and obesity.	1
Small size and mentally handicapped.	1
Convulsions.	2
Headaches.	3
Headaches and fits.	1
Failure to thrive and recurrent cough.	1
Persistent cough, obesity and constipation.	1
Failure to thrive and offensive stools.	1
Anorexia, sickness and diarrhoea.	1
Vomiting and failure to thrive.	1
Undescended testes and small penis.	1
Undescended testes.	1
Excessive thirst and polyuria.	1
Eczema.	2
Anaemia.	1
	41

GIRLS

Chief complaint	Number of patients
Small size.	15
Poor appetite and failure to gain weight.	5
Failure to thrive and convulsions.	4
Physical and mental retardation.	3
Eczema.	1
Anaemia and epistaxis.	1
	29

Table 2.

BIRTH WEIGHT

BOYS (See Fig. 2)

Patient	Weight	No. of Pregnancy	Pregnancy and Confinement
2	8 lb 4 oz	3	Normal, full-term, spontaneous vertex delivery.
3	7 lb	1	Normal, full-term, spontaneous vertex delivery.
4	4 lb 1½ oz	2	Mild toxaemia. 6 weeks premature. Spontaneous vertex delivery.
5	3 lb 8 oz	1	Normal pregnancy. 6 weeks premature. Breech delivery.
6	8 lb 4 oz	4	Normal, full-term, spontaneous vertex delivery.
7	4 lb	1	Abdomen small during pregnancy. Normal, full-term, spontaneous vertex delivery.
8	6 lb 4 oz	2	Normal, full-term, spontaneous vertex delivery.
9	4 lb 5 oz	3	Normal, one week postmature, spontaneous delivery.
10	6 lb 7 oz	2	Normal, full-term, spontaneous vertex delivery.
11	6 lb 11 oz	1	Normal, full-term, spontaneous vertex delivery.
12	6 lb 4 oz	2	Normal, full-term, spontaneous vertex delivery.
13	9 lb	1	Lower segment Caesarean section for old fracture of pelvis.
14	4 lb 14 oz	1	Normal, full-term, spontaneous vertex delivery.
15	9 lb 2 oz	2	Normal, full-term, spontaneous vertex delivery.
16	8 lb	1	Normal, full-term, spontaneous vertex delivery.

(Continued)

BIRTH WEIGHT

BOYS

(Continued)

Patient	Weight	No. of Pregnancy	Pregnancy and Confinement
17	5 lb 6½ oz	1	Mild toxaemia, full-term, spontaneous vertex delivery. Rapid 2nd stage. "Asphyxia livida" twitchings after birth.
18	5 lb 14 oz	2	42 weeks duration. Spontaneous vertex delivery.
19	Not stated	1	Not stated.
20	6 lb 5 oz	2	Normal, full-term, spontaneous vertex delivery.
21	7 lb	1	Small haemorrhage for 5 days during 1st month. Spontaneous vertex delivery.
22	7 lb	1	1 month premature. Spontaneous vertex delivery.
23	6 lb 4 oz	1	Normal, full-term, spontaneous vertex delivery. Abdomen "small during pregnancy".
24	7 lb 14 oz	1	Normal, full-term, spontaneous vertex delivery.
25	4 lb 8 oz	2	Toxaemia, Caesarean section at 7 months.
26	3 lb 6 oz	1	? full-term. Abdomen small during pregnancy. Doctor uncertain at 4 months. Very poor intra-uterine movements. First stage - 3 days, second stage - 2 hours. Very small placenta. Cyanosis at birth.
27	3 lb 10 oz	3	Premature birth at 30-31 weeks gestation. Normal delivery.
28	8 lb	4	Normal, full-term, spontaneous vertex delivery.
29	5 lb 14 oz	1	Normal, full-term, spontaneous vertex delivery. Post-partum haemorrhage.

(Continued)

BIRTH WEIGHT

BOYS

(Continued)

Patient	Weight	No. of Pregnancy	Pregnancy and Confinement
30	7 lb	2	Normal, full-term, spontaneous vertex delivery. Breech presentation.
31	7 lb 8 oz	8	Normal, full-term, spontaneous vertex delivery.
32	6 lb 5 oz	1	Normal, full-term, spontaneous vertex delivery.
33	6 lb 1 oz	2	Normal, full-term, spontaneous vertex delivery.
34	8 lb 4 oz	4	Normal, full-term, spontaneous vertex delivery.
35	2 lb 8 oz	9	2 months premature, spontaneous delivery. Smallest and sole survivor of triplets.
36	5 lb 13 oz	1	Full-term, 36 hour labour, forceps delivery for foetal distress.
37	Not stated	3	Normal, full-term, spontaneous delivery.
38	7 lb 4 oz	1	Normal, full-term, spontaneous vertex delivery.
39	7 lb 15¾ oz	1	43 weeks duration, 3 day labour, forceps delivery.
40	4 lb	4	Toxaemia. ? occipito-posterior position. 2 weeks premature by dates.
41	7 lb	2	Hyperemesis gravidarum. 8 months gestation. Breech presentation. Forceps delivery. General anaesthesia. Shock and cyanosis at birth.

Table 3.

BIRTH WEIGHT

GIRLS (See Fig. 3)

Patient	Weight	No. of Pregnancy	Pregnancy and Confinement
2	4 lb 2 oz	2	1 week post-mature by dates. Spontaneous vertex delivery.
3	4 lb 13 oz	2	Not premature by dates. Caesarean section for uterine inertia first pregnancy.
4	5 lb	2	Normal, full-term, spontaneous vertex delivery. Poor condition at birth.
5	7 lb 4 oz	1	Normal, full-term, spontaneous vertex delivery.
6	7 lb 11 oz	5	Normal, full-term, spontaneous vertex delivery.
7	6 lb	5	Normal, full-term, breech delivery.
8	6 lb	1	Normal, full-term, spontaneous vertex delivery.
9	7 lb 12 oz	2	Normal, full-term, spontaneous vertex delivery.
10	5 lb	1	Normal, 10 days postmature, spontaneous vertex delivery.
11	5 lb 12 oz	Adopted	
12	7 lb 4 oz	1	Normal, full-term, spontaneous vertex delivery.
13	6 lb	3	Normal, full-term, spontaneous vertex delivery.
14	6 lb 5 oz	3	Salmonella infection at 3 months. 43 weeks duration. Face presentation.
16	4 lb	Not stated	Severe ante-partum haemorrhage at 2 months. 8 month pregnancy. Spontaneous vertex delivery.
17	5 lb 14 oz	1	Normal, full-term, spontaneous vertex delivery.

(Continued)

BIRTH WEIGHT

GIRLS

(Continued)

Patient	Weight	No. of Pregnancy	Pregnancy and Confinement
18	10 lb 8 oz	Not stated	Normal, full-term, spontaneous vertex delivery.
19	3 lb	1	Normal, full-term, breech presentation, forceps delivery.
20	8 lb	2	Normal, full-term, spontaneous vertex delivery.
21	3 lb 9 oz	2	8 month pregnancy, spontaneous vertex delivery. Oxygen given for first 24 hours.
22	4 lb 11 oz	2	Normal, full-term, twin pregnancy. Spontaneous delivery. Twin still-born, weight 5 lb. 8 oz. Normal, full-term, spontaneous vertex delivery.
25	5 lb 12 oz	3	Normal, full-term, spontaneous vertex delivery.
26	6 lb 4 oz	2	Normal, 2 weeks premature, spontaneous delivery.
27	4 lb 8 oz	2	External cephalic version, breech presentation. Ante-partum haemorrhage followed by surgical induction.
28	4 lb 2 oz	2	Normal, full-term, spontaneous "rapid" vertex delivery.
29	7 lb 1 oz	3	Mild toxaemia. 10 days postmature by dates. External version. Spontaneous vertex delivery.

Table 4.

MATERNAL AGE AT PATIENT'S BIRTH

BOYS

Patient	Maternal age at patient's birth (years)
2	31
3	30
4	31
5	23
7	24
9	23
11	27
17	32
18	31
21	26
22	23
23	20
24	43
25	33
26	30
28	26
29	20
30	40
31	31
32	24
33	24
34	25
35	41
36	19
38	26

Table 5.

MATERNAL AGE AT PATIENT'S BIRTH

GIRLS

Patient	Maternal age at patient's birth (years)
3	26
4	34
5	28
7	35
8	19
9	30
10	22
11	32
14	30
17	23
19	20
20	25
22	24
23	28
25	25
27	28

SKELETAL AGE

Skeletal age is a radiological measure of the developmental status of the bones and it has been the most commonly used indicator of physiological maturity (Tanner, 1962). Although the assessment is approximate, it is of particular value in clinical paediatrics and its diagnostic use in relation to endocrinology has been described (Mellman, Bongiovanni and Hope, 1959).

The method used in the present study was that described by Greulich and Pyle (1959), in which a given X-ray was compared with standards derived from a very large series of children. Briefly, a film of the left wrist was compared with the standard for the same sex and nearest chronological age in the Atlas. The film was then matched with adjacent standards which appeared to resemble it most closely on superficial examination. A more detailed study was next made of each individual bone, and lastly, a mean value was assigned to the entire wrist and hand.

All assessments were made on the left hand in accordance with established custom. According to Greulich and Pyle (1959), except in grossly pathological states the difference between the right and left side is negligible when one compares the entire wrist and hand.

It has already been stated that assessments of skeletal age are approximate. The application of more rigorous standards in

this country must await publication of those values for skeletal maturity which are based upon a study of three thousand British children (Tanner, Whitehouse and Healy, 1961). Ellis (1962), has written that Greulich and Pyle standards, which were founded upon North American data, are not strictly applicable to other populations and he stated that allowance must also be made for secular change.

The precision of the Greulich-Pyle standards demands exact positioning of the hand for X-ray and the assessment of each film is open to subjective error. Thus, Mainland (1953,1954), has shown that the consistency of performance of a single observer is such that only two-thirds of duplicate assessments fall within ± six months. Yet another source of error is found in the rating of different bones in the wrist which show different skeletal ages in relation to the standards. There is no agreement as to how much significance should be attached to each bone for the overall assessment.

Table 6.

PARENTAL HEIGHTS

BOYS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Father's height (inches)	Mother's height (inches)
3	10.53	7y 2m	67	66
4	3.74	1y 4m	63	58
6	8.52	5y 0m	67.5	64
7	4.48	3y 3m	71.5	66.5
9	2.15	0y 10m	64.75	61.5
10	2.49	1y 10m	66	64
11	5.92	2y 6m	66.5	62
12	10.32	8y 0m	68.5	64
16	1.17	0y 6m	75	64
18	8.08	4y 2m	69	62
19	11.94	7y 6m*	-	60
21	2.42	0y 7½m	69	67
22	14.07	9y 0m	72	65
23	7.6	4y 0m	69	62
24	11.06	7y 6m	61.5	61.5
25	14.02	10y 9m	72	68.5
28	14.5	8y 3m	63.75	61
29	13.43	8y 0m	60	64.5
31	8.1	4y 6m	-	64
32	3.65	2y 7m	68	61
33	4.98	1y 9m	68	60
35	10.0	6y 3m	62	62
36	7.38	5y 1m	71.5	61
38	7.59	4y 5m	71	67
39	9.56	4y 9m	70	61
41	13.25	8y 9m	68	68

* Estimated by Dr. M.E.Sidaway.

Table 7.

PARENTAL HEIGHTS

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Father's height (inches)	Mother's height (inches)
2	1.31	10½m	70	61
3	2.75	1y 11m	67	58
6	4.92	2y 9m	63	63
8	6.66	4y 9m	63	62
9	2.0	1y 4m	70	64
10	4.99	3y 9m	65	59.5
12	6.75	3y 6m	68	61
16	9.92	6y 4m	63	61
17	6.67	5y	64	61.5
19	3.58	2y 5m	71	59
20	9.52	4y	64.5	62.8
22	11.98	6y 8m	72	64
23	3.91	2y 4m	66	64
25	1.5	1y 5m	62	62
26	9.25	-	68.5	65.5
27	16.55	11y	72	60
28	8.63	7y 6m	67	62
29	2.33	2y 2m	67	61.5

Table 8.

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR SKELETAL AGE

BOYS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
2	14.45	11y 0m	149.1	90	40.9	95
3	10.53	7y 2m	124.0	70	24.8	80
4	3.74	1y 4m	77.6	30	7.0	<3
5	2.08	1y 3m	80.0	70	8.75	<3
6	8.52	5y 0m	114.4	Just above 90	19.72	Just below 75
7	4.48	3y 3m	91.9	17	11.18	<3
8	13.92	14y 0m	133.75	<3	31.16	<3
9	2.15	0y 10m	79.3	>97	9.1	25
10	2.49	1y 10m	82.2	Just below 25	9.9	4
11	5.92	2y 6m	85.3	7	11.1	5
12	10.32	8y 0m	108.8	<3	19.7	5
13	14.6	14y 0m	150.8	Just below 25	43.21	45
14	2.25	9-12m	81.2	<97	7.8	>3
16	1.17	0y 6m*	65.9	40	6.65	12
18	8.08	4y 2m	108.75	Just above 90	17.3	80
19	11.94	7y 6m*	128.75	85	42.56	>97
20	8.25	5y 0m*	105.9	35	18.29	45
21	2.42	7.5m	68.3	40	7.7	20
22	14.07	9y 0m	120.5	6	24.5	20
23	7.6	4y 0m	105.2	80	17.1	60
24	11.06	7y 6m	111.2	<3	22.7	37

* Estimated by Dr. M.E.Sidaway.

< = less than. > = more than.

(Continued)

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR SKELETAL AGE

BOYS

(Continued)

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
25	14.02	10y 9m	136.5	35	43.9	> 97
28	14.5	8y 3m	130.6	75	25.6	60
29	13.43	8y 0m	128.1	70	42.7	> 97
30	7.76	9y 3m	123.6	7	25.8	Just below 25
31	8.1	4y 6m	99.8	Just above 10	14.4	7
32	3.65	2y 7m	83.4	< 3	9.2	< 3
33	4.98	1y 9m	78.8	7	8.7	< 3
34	7.75	2y 6m*	91.25	45	17.27	> 97
35	10.0	6y 3m	115.5	50	16.7	4
36	7.38	5y 1m	103.1	10	18.86	50
37	2.79	0y 9m	81.8	> 97	11.2	94
38	7.59	4y 5m**	113.1	97	18.86	75
39	9.56	4y 9m	110.7	82	22.5	> 97
40	15.28	10y 0m	131.3	Just below 25	39.8	> 97
41	13.25	8y 9m	133.4	Just above 75	33.2	96

* Estimated by Dr. J.S.Sutcliffe at chronological age 7.33 years.

** Estimated two weeks after height and weight.

< = less than. > = more than.

Table 9.

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR SKELETAL AGE

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
1	7.08	3y 3m	87.5	Just below 3	10.76	< 3
2	1.31	10½m	70.0	25	7.6	7
3	2.75	1y 11m	78.1	Just below 3	8.5	< 3
4	5.5	3y 3m	-	-	17.2	93
5	13.11	10y	125.4	7	23.3	4
6	4.92	2y 9m	96.3	94	14.7	77
8	6.66	4y 9m	102.5	23	16.1	24
9	2.0	1y 4m	76.25	25	10.0	37
10	4.99	3y 9m	97.3	37	12.1	< 3
11	13.17	8y 3m	125.8	50	25.9	67
14	1.66	11m	74.0	75	8.21	16
16	9.92	6y 4m	115.9	Just above 50	21.08	65
17	6.67	5y	100.5	7	13.6	< 3
18	8.79	2y 4m	109.4	> 97	28.1	> 97
19	3.58	2y 5m	87.0	30	9.5	< 3
20	9.52	4y	93.0	3	14.2	11
21	12.22	10y 6m	127.5	7	22.9	< 3
22	11.98	6y 8m	111.3	22	19.1	17
23	3.91	2y 4m	80.6	< 3	10.68	8
25	1.5	1y 5m	83.1	95	10.9	55
27	16.55	11y	128.6	4	29.8	23
28	8.63	7y 6m	115.4	12	18.6	Just above 3
29	2.33	2y 2m	76.6	< 3	8.7	< 3

< = less than. > = more than.

Table 10.

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR CHRONOLOGICAL AGE

BOYS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
2	14.45	11y 0m	149.1	Just below 10	40.9	17
3	10.53	7y 2m	124.0	<3	24.8	4
4	3.74	1y 4m	77.6	<3	7.0	<3
5	2.08	1y 3m	80.0	<3	8.75	<3
6	8.52	5y 0m	114.4	<3	19.72	<3
7	4.48	3y 3m	91.9	<3	11.18	<3
8	13.92	14y 0m*	133.75	<3	31.16	<3
9	2.15	0y 10m**	79.3	<3	9.1	<3
10	2.49	1y 10m	82.2	<3	9.9	<3
11	5.92	2y 6m	85.3	<3	11.1	<3
12	10.32	8y 0m	108.8	<3	19.7	<3
13	14.6	14y 0m	150.8	Just below 10	43.21	<3
14	2.25	9-12m	81.2	<3	7.8	<3
15	9.91	-	108.4	<3	27.95	30
16	1.17	0y 6m***	65.9	<3	6.65	<3
17	6.16	-	112.8	30	20.31	48
18	8.08	4y 2m	108.75	<3	17.3	3
19	11.94	7y 6m***	128.75	<3	42.56	91
20	8.25	5y 0m***	105.9	<3	18.29	<3
21	2.42	7.5m	68.3	<3	7.7	<3
22	14.07	9y 0m	120.5	<3	24.5	<3
23	7.6	4y 0m	105.2	<3	17.1	<3
24	11.06	7y 6m	111.2	<3	22.7	<3

* Estimated by Dr. J.S.Sutcliffe 20 weeks after height and weight, and after steroid therapy.

** Estimated one week before height and weight.

*** Estimated by Dr. M.E.Sidaway.

(Continued)

< = less than. > = more than.

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR CHRONOLOGICAL AGE

BOYS

(Continued)

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
25	14.02	10y 9m	136.5	<3	43.9	Just below 50
26	1.33	"Under 3m"	66.25	<3	5.4	<3
27	5.66	-	98.75	<3	14.68	<3
28	14.5	8y 3m	130.6	<3	25.6	<3
29	13.43	8y 0m	128.1	<3	42.7	70
30	7.76	9y 3m	123.6	45	25.8	Just above 75
31	8.1	4y 6m	99.8	<3	14.4	<3
32	3.65	2y 7m	83.4	<3	9.2	<3
33	4.98	1y 9m	78.8	<3	8.7	<3
34	7.75	2y 6m*	91.25	<3	17.27	<3
35	10.0	6y 3m	115.5	<3	16.7	<3
36	7.38	5y 1m	103.1	<3	18.86	6
37	2.79	0y 9m	81.8	<3	11.2	<3
38	7.59	4y 5m**	113.1	3	18.86	4
39	9.56	4y 9m	110.7	<3	22.5	4
40	15.28	10y 0m	131.3	<3	39.8	4
41	13.25	8y 9m	133.4	<3	33.2	7

* Estimated by Dr. J.S.Sutcliffe at chronological age 7.33 years.

** Estimated two weeks after height and weight.

< = less than. > = more than.

Table 11.

HEIGHT AND WEIGHT

PERCENTILE RATINGS FOR CHRONOLOGICAL AGE

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Height (cm.)	Percentile rating (height)	Weight (kg.)	Percentile rating (weight)
1	7.08	3y 3m	87.5	<3	10.76	<3
2	1.31	10½m	70.0	<3	7.6	<3
3	2.75	1y 11m	78.1	<3	8.5	<3
4	5.5	3y 3m	-	-	17.2	17
5	13.11	10y	125.4	<3	23.3	<3
6	4.92	2y 9m	96.3	<3	14.7	5
7	2.33	-	80.5	<3	9.9	<3
8	6.66	4y 9m	102.5	<3	16.1	<3
9	2.0	1y 4m	76.25	<3	10.0	6
10	4.99	3y 9m	97.3	<3	12.1	<3
11	13.17	8y 3m	125.8	<3	25.9	<3
12	6.75	-	95.6	<3	16.7	<3
13	13.5	-	116.7	<3	26.36	<3
14	1.66	11m	74.0	<3	8.21	<3
16	9.92	6y 4m	115.9	<3	21.08	<3
17	6.67	5y	100.5	<3	13.6	<3
18	8.79	2y 4m	109.4	<3	28.1	75
19	3.58	2y 5m	87.0	<3	9.5	<3
20	9.52	4y	93.0	<3	14.2	<3
21	12.22	10y 6m	127.5	<3	22.9	<3
22	11.98	6y 8m	111.3	<3	19.1	<3
23	3.91	2y 4m	80.6	<3	10.68	<3
25	1.5	1y 5m	75.3	<3	8.47	<3
26	9.25	-	106.9	<3	18.57	<3
27	16.55	11y	128.6	<3	29.8	<3
28	8.63	7y 6m	115.4	<3	18.6	<3
29	2.33	2y 2m	76.6	<3	8.7	<3

< = less than. > = more than.

Table 12.

AGE AT WHICH SMALL SIZE WAS FIRST OBSERVED

BOYS

Age	Patient
0-1 year	3, 4, 5, 7, 9, 10, 11, 12, 14, 15, 16, 18, 21, 26, 29, 32, 33, 35, 36, 39.
1-2 years	37, 40.
2-3 years	24, 25, 34, 41.
3-4 years	6, 22, 31.
4-5 years	23.
5-6 years	20.
Normal at 5 years, small at 9 years.	19.
12 years	28.
Small size noted incidentally on examination.	2, 8, 13, 17, 27, 30, 38.
	Total number of patients = 40

GIRLS

Age	Patient
0-1 year	2, 4, 10, 11, 13, 14, 16, 17, 19, 20, 21, 22, 25, 26, 27, 29.
1-2 years	6, 9, 28.
2-3 years	3, 8.
3-4 years	12, 18.
7-8 years	5.
	Total number of patients = 24

THE INSULIN TOLERANCE TEST

Fraser, Albright and Smith (1941) discussed the value of the intravenous insulin tolerance test in the diagnosis of endocrine disorders of carbohydrate metabolism and they stated that the test had two purposes:-

a. to measure the sensitivity of the blood glucose level to insulin,

b. to measure the "responsiveness" of the blood glucose level to an insulin-induced hypoglycaemia.

It was concluded that the test was of value in differentiating pan-hypopituitarism from some cases of anorexia nervosa and from primary hypothyroidism.

McQuarrie (1954) upheld the value of the investigation particularly in the study of hypoglycaemia in children, but Martin and Wilkins (1958) wrote that they had not employed the insulin tolerance test for some years because of the danger inherent in the intravenous use of insulin and because of the difficulty in the interpretation of the results. The hazards of intravenous insulin are not denied but children respond to the test in the same manner as adults even when the insulin is given subcutaneously. Clayton (1962), with experience of some two hundred investigations, has found the subcutaneous insulin tolerance test to be a valuable screening procedure in suspected

hypofunction of the anterior pituitary or adrenal cortex, provided the child is under constant nursing surveillance.

Procedure:- The method is that of Daniel (1941), which as he claimed is relatively simple to perform and which obviates the need for expensive apparatus. The child is tested in the fasting state preferably in the morning. The diet prior to the test should be the normal one for the age in so far as the clinical condition will permit (Himsworth, 1939). After the collection of a fasting blood sample by heel or finger-prick, a standard dose of 0.25 units of soluble insulin per kilogram of body-weight is injected subcutaneously. Further blood samples are taken at 20, 40, 60 and 120 minutes after the administration of the hormone and total blood reducing substances are estimated by micro-method (Wilkinson, 1960). In children of short stature or in suspected cases of hypopituitarism or hypoglycaemia, 0.125 units of soluble insulin are given per kilogram of body-weight and this is called an "half-strength" tolerance. The child is under constant nursing supervision throughout the test, and if the clinical condition demands the investigation is stopped by the administration of oral glucose after the collection of a blood sample. In this series only one child required the administration of intravenous glucose.

Interpretation:- The interpretation of the test lies in the form of the curve. Following the administration of insulin, in

THE INSULIN TOLERANCE TEST

TYPICAL RESPONSES

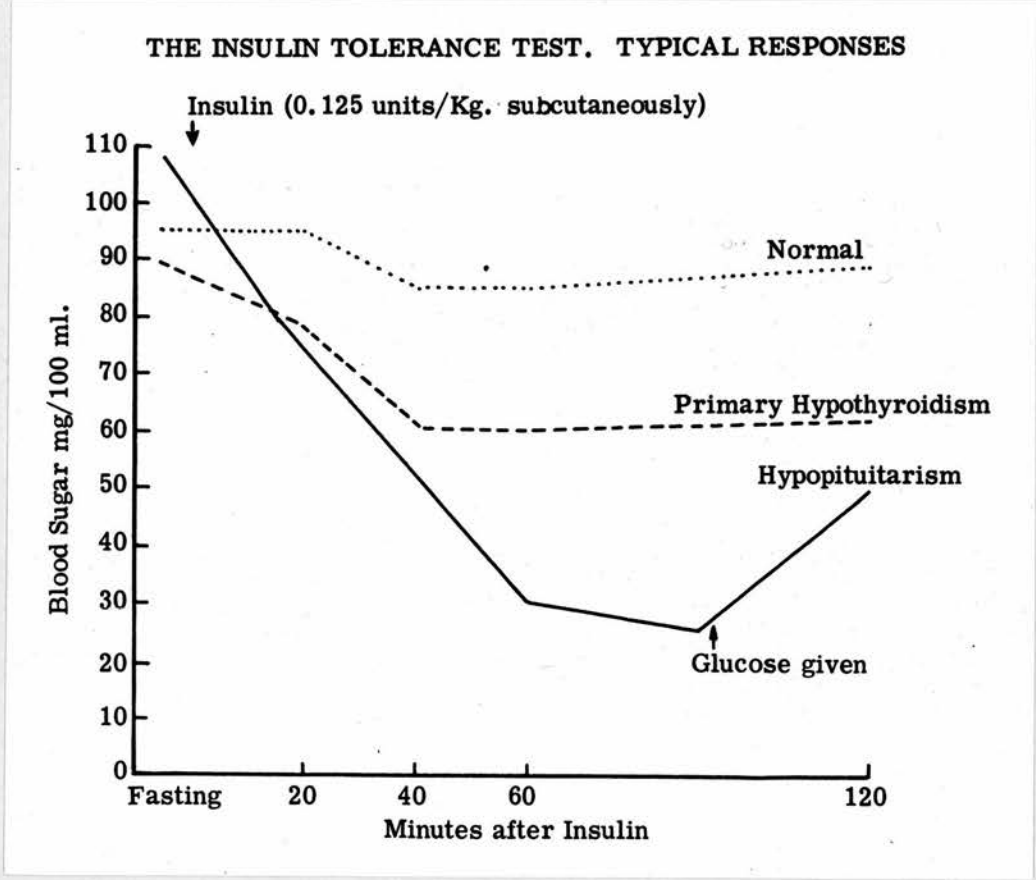

Fig. 1.

the healthy child the blood sugar level falls during the first thirty minutes, and reaches the lowest value by the end of the first hour, returning to fasting level by the 120 minute sample. The difference between the fasting blood sugar and its lowest level ranges from 15 - 25 mg./100 ml. (see Fig. 1).

Fraser and Smith (1941) expressed "hypoglycaemia unresponsiveness" quantitatively, by measuring the slope of the curve after the maximal fall had been reached; this is unnecessary. These authors also found that to a limited extent, the degree of failure to respond to hypoglycaemia corresponded to the degree of pituitary failure on clinical assessment.

An excessively steep fall in blood sugar with a slow return or a failure to achieve fasting values is seen in adrenal or pituitary insufficiency, starvation, glycogen storage disease, galactosaemia and gastro-intestinal disturbance with poor absorption of glucose (Hartmann and Jaudon, 1937).

Excepting the newborn, the average normal response is almost the same in infants and older children. Healthy newborn infants with physiological hypoglycaemia have a low tolerance (Hartmann and Jaudon, 1937).

The results of the tests are given in Tables 13 to 16, inclusive.

Table 13.

"HALF-STRENGTH" INSULIN TOLERANCE

BOYS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.)				
			Fasting	Minutes after insulin: 20	40	60	120
1	7.0	-	122	79	74	68	68
2	14.45	11y 0m	137	79	50	58	47
4	3.74	1y 4m	87	83	63	84	64
5	2.08	1y 3m	95	59	50	59	55
6	8.52	5y 0m	73	85	80	70	75
7	4.48	3y 4m	81	81	66	69	75
8	13.92	14y 0m*	100	91	69	65	50
9	2.15	0y 10m**	90	63	50	38	54
10	2.49	1y 10m	81	81	76	81	71
11	5.92	2y 6m	81	75	56	69	69
12	10.32	8y 0m	100	113	62	62	69
13	14.6	14y 0m	89	87	87	89	89
14	1.75	0y 6m	78	78	70	85	75
15	13.75	-	88	76	58	58	61
16	1.17	0y 6m***	63	42	45	45	32
17	6.16	-	95	84	71	50	50
18	8.08	4y 2m	112	82	76	89	106
19	11.94	7y 6m***	67	62	62	60	55
20	8.25		92	100	82	66	74
21	2.42	0y 7½m	48	26	24	16****	21
22	13.08	8y 0m	95	83	68	66	72
23	7.6	4y 0m	92	83	72	67	56

* Estimated by Dr. J.S.Sutcliffe 20 weeks after height and weight, and after steroid therapy.

** Estimated one week before height and weight.

*** Estimated by Dr. M.E.Sidaway.

**** Intravenous glucose given after 60 minute sample.

(Continued)

"HALF-STRENGTH" INSULIN TOLERANCE

BOYS

(Continued)

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.)				
			Fasting	Minutes after insulin: 20	40	60	120
25	14.02	10y 9m	70	65	60	65	65
27	5.58	-	78	75	66	53	30
28	14.5	8y 3m	84	84	69	71	106
29	13.43	8y 0m	78	78	72	69	69
30	6.83	7y 0m	90	87	76	76	76
31	8.1	4y 6m	83	66	48	57*	48
32	3.65	2y 7m	90	74	53	50	47
33	4.98	1y 9m	56	38	44	38	25
34	7.75	2y 6m**	75	48	35	33	33
35	10.0	6y 3m	100	85	75	72	69
36	7.38	5y 1m	118	82	67	67	63
37	2.79	0y 9m	95	105	81	58	63
38	7.59	4y 5m	89	79	81	73	68
39	9.56	4y 9m	87	86	84	77	75
40	15.28	10y 0m	83	71	79	71	69
41	13.82	9y 6m	100	76	28	39*	-

* Oral glucose given after 60 minute sample.

** Estimated by Dr. J.S.Sutcliffe at chronological age 7.33 years.

Continued.

"HALF-STRENGTH" INSULIN TOLERANCE

BOYS

(Continued)

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.) Minutes after insulin: Fasting	15	30	45	60	120
3*	9.3	5y 6m	96	87	87	84	84	89
24	11.1	7y 6m	108	80	63	-	29*	50

* Full-strength insulin tolerance.

"HALF-STRENGTH" INSULIN TOLERANCE

BOYS

Patient	Chronological age (years)	Skeletal age	Blood sugars (mg./100 ml.) Minutes after insulin: Fasting	25	60	90	120
26	1.33	"Under three months"	110**	52	36	42	40

* Oral glucose given after 60 minute sample.

** Fasting blood sugar was estimated on the following day.

Table 14.

"FULL-STRENGTH" INSULIN TOLERANCE

BOYS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.)				
			Fasting	Minutes after insulin: 20	40	60	120
4	3.74	1y 4m	86	63	56	52	54
6	8.52	5y 0m	116	95	50	85	50
7	4.48	3y 3m	81	81	69	69	60
13	14.6	14y 0m	88	86	64	59	46
14	1.75	0y 6m	81	58	69	69	66
15	13.75	-	89	89	68	63	63
18	8.08	4y 2m	89	74	58	58	56
25	14.02	10y 9m	83	86	77	80	74
28	14.5	8y 3m	88	68	60	60	58
29	13.43	8y 0m	80	80	75	68	68
37	2.79	0y 9m	90	42	50	50	65
38	7.59	4y 5m	89	78	53	60	53
39	9.56	4y 9m	88	78	50	53	53

Table 15.

"HALF-STRENGTH" INSULIN TOLERANCE

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.) Minutes after insulin: Fasting	20	40	60	120
1	7.08	3y 3m	69	56	41	38	44
3	3.81	1y 11m	95	77	74	72	82
4	5.54	3y 3m	66	55	55	52	47
5	13.11	10y 0m	95	95	69	69	62
6	4.92	2y 9m	78	61	48	45	40
7	2.33	-	100	100	-	76	88
8	6.66	4y 9m	80	60	52	63	57
9	1.96	1y 0m	86	76	65	60	57
10	4.99	3y 9m	95	90	80	85	85
11	13.17	8y 3m	81	81	65	65	70
12	6.75	3y 6m*	97	84	79	81	66
13	13.5	10y 0m**	83	88	81	86	91
14	1.89	0y 11m	100	74	58	68	58
15	4.56	"Grossly retarded"	45	39	30	28	34
16	9.92	6y 2m	69	81	75	67	58
17	6.66	5y 5m	91	88	82	82	76
18	8.91	1y 8m	89	78	61	61	63
19	3.58	2y 5m	79	79	66	66	65
20	9.52	4y 0m	42	35	25	25***	-
21	12.22	10y 6m	105	90	85	80	80
22	11.98	6y 8m	106	100	94	100	75
23	1.5	0y 10m	95	95	86	86	88
24	11.17	"Retarded"	84	95	63	63	58
25	1.51	1y 4m	60	55	45	50	50
26	8.6	About 5y**	138	126	130	130	132

* Estimated by Dr. R.D.Hoare.

** Estimated by Dr. M.E.Sidaway.

*** Oral glucose given after 60 minute sample.

(Continued)

"HALF-STRENGTH" INSULIN TOLERANCE

GIRLS

(Continued)

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.)				
				Minutes after insulin:			
			Fasting	20	40	60	120
27	16.55	11y 0m	89	86	62	68	65
28	8.63	7y 6m	86	79	76	73	73
29	2.33	2y 2m	82	57	62	68	55

"FULL-STRENGTH" INSULIN TOLERANCE

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.) Minutes after insulin:				
			Fasting	20	40	60	120
3	2.75	1y 11m	63	74	84*	132	58
7	2.33	-	95	62	60	53	50
10	4.99	3y 9m	91	95	91	63	63
12	6.75	3y 6m	91	86	52	58	55
21	12.22	10y 6m	82	83	-	67	61
23	3.91	2y 4m	100	62	54	50	62
26	8.5	5y 0m	152	120	116	128	116
28	8.63	7y 6m	90	80	75	70	83

"FULL-STRENGTH" INSULIN TOLERANCE

GIRLS

Patient	Chronological age (years)	Skeletal age (y = yrs) (m = mths)	Blood sugars (mg./100 ml.) Minutes after insulin:					
			Fasting	15	30	45	60	120
11	13.17	8y 3m	100	97	75	72	69	65
13	13.0	10y 0m**	93	93	80	80	84	80

* Drank glucose before 40 minute sample.

** Estimated by Dr. R.D.Hoare.

TESTS OF ADRENAL CORTICAL FUNCTION AND INTEGRITY OF THE PITUITARY ADRENAL AXIS

The clinical diagnosis of adrenal cortical hypofunction may be difficult to establish because features such as anorexia, recurrent dehydration and general debility are not specific for this condition (Talbot, Sobel, McArthur and Crawford, 1952). Often the diagnosis is made in retrospect or after the child has been under clinical observation for several years (Greenberg, 1958).

Among the laboratory investigations which are used as tests of adrenal cortical function, several are indirect and they include the measurement of:-

1. Plasma and urinary electrolytes and blood urea.
2. Absolute eosinophil counts.
3. Ability to excrete a water-load.
4. Insulin tolerance.

In mild degrees of hypofunction, 1 and 2 may be normal. A water-load test in a patient with suspected adrenal deficiency is potentially dangerous since water intoxication may result. Also, such a test may be invalid in the young child who cannot empty his bladder to order. Insulin tolerance, although useful and safe in children if performed by the subcutaneous route (Daniel, 1941; Clayton, 1962) is not specific for adrenal cortical deficiency.

Chemical methods for assessing adrenal cortical function have been applied frequently in adults (Nelson, Samuels, Willardson and Tyler, 1951; Gordon, Horwitt and Segaloff, 1954), and patterns of steroid response to adrenocorticotrophic hormone (ACTH) stimulation have been clearly defined in various pathological states (Prunty, 1956).

In children, as in adults (Prunty, 1956), there is considerable day to day variation in the excretion of steroids. Since the urinary excretion of 17-ketosteroids and 17-hydroxycorticoids is so low in children (Norymberski, Stubbs and West, 1953; Prout and Snaith, 1958), it is unwise to diagnose defective adrenal cortical function by the measurements of resting steroid excretions. It has been observed in adults that zero levels of urinary steroids and blood corticoids are frequently not encountered in Addison's Disease (Laidlaw, Reddy, Jenkins, Haydar, Renold and Thorn, 1955; Bayliss, 1955). It is, therefore, necessary to measure the response of the adrenal gland to injected ACTH in an attempt to demonstrate degrees of adrenal failure.

The test has been employed in several ways in children. Gottfried, Bogin and Levycky (1957) gave one injection of ACTH in gelatin solution at 10 p.m. and determined the urinary excretion of 17-hydroxycorticoids during the following eight hours. Although a definite rise in steroid excretion was obtained in normal children, the response was, in fact, so small that it would be difficult to detect anything other than complete adrenal failure.

Ely, Raile, Bray and Kelley (1954) measured the 17-hydroxycorticoids in the plasma before and after ACTH. The values obtained showed a wide variation between individuals, and the authors considered that rigid interpretation of the results was unwise as some normal children gave a poor response. The results obtained by Steiker, Bongiovanni, Eberlein and Leboeuf (1961), employing a similar type of test, were more regular and impaired adrenal responses could be diagnosed.

Clayton, Edwards and Renwick (1962) employed three days' stimulation with ACTH, since it may take several days to elicit a near maximal response (Prunty, 1956). The urinary excretion of corticosteroids was studied in preference to blood because blood may not be collected at the peak of the response, and diurnal variations in 17-hydroxycorticoid levels may affect the size of the response. In addition, no satisfactory micro-method using finger-prick blood is yet available for the determination of 17-hydroxycorticoids.

Method of Performing the ACTH Test:

The test was begun at 10 a.m. Four consecutive 24-hour urine specimens were collected using a few drops of chloroform as preservative. Twenty physiological units of ACTH gel (H.P. Acthar gel, Armour Laboratories Ltd.) were administered intramuscularly at 10 a.m. and 6 p.m. on the second, third and fourth days. In the course of several hundred tests no unpleasant side-effect was ever observed.

Two batches of ACTH of porcine origin were employed:-

1. CK 2501 Initial potency 40 I.U./ml.
   Limits = 37.1 - 42.9 I.U./ml.
   Fiducial limits (P = 0.95) 87 - 115%.

2. EE 1004 Initial potency 38.4 I.U./ml.
   Limits = 34.8 - 42.4 I.U./ml.
   Fiducial limits (P = 0.95) 87 - 106%.

Hence 20 units were given in 0.5 and 0.52 ml. gel of batches CK 2501 and EE 1004 respectively; a tuberculin syringe was used for injection.

Urinary 17-ketosteroids were determined by the second method of Prout and Snaith (1958) and total 17-hydroxycorticoids by the method of Appleby, Gibson, Norymberski and Stubbs (1955).

Interpretation:

In thirty normal children investigated by Clayton, Edwards and Renwick (1962), no variation in response was seen with age, and the eight girls in the group did not give responses significantly different from those of the boys; these observations confirmed the work of Steiker et al. (1961). As shown by others (e.g. Gottfried et al., 1957), the total

17-hydroxycorticoids were a more sensitive index than the 17-ketosteroids and in the routine investigation there would appear to be no additional benefit from estimating the 17-ketosteroids. The maximal corticoid excretion occurred during the second or third days of ACTH administration and, with one exception, always reached at least 20 mg./24 hours on one or other of those days.

The normal values employed in this laboratory for 17-ketosteroids and total 17-hydroxycorticoids are as follows:-

17-ketosteroids (Prout and Snaith, 1958)

0-1 year: 0.25 ± 0.12 mg./24 hours (± standard deviation).

1-5 years: 0.78 ± 0.46 mg./24 hours.

6-10 years: 1.38 ± 0.74 mg./24 hours.

11-17 years: 4.96 ± 2.06 mg./24 hours.

Total 17-hydroxycorticoids (Appleby, Gibson, Norymberski and Stubbs, 1955).

There is a gradual rise during childhood, with a sharp rise at puberty.

Up to 10 years: 1.0 - 4.5 mg./24 hours.

10 - 20 years: 4.5 - 19 mg./24 hours, depending on age, sex and body build.

In this series of investigations, plasma electrolytes and blood urea were determined on capillary samples before the administration of ACTH and on the fourth day of the test. Normal values (Wilkinson, 1960) are listed below:-

Sodium:	138 - 143 m.Eq./L.
Potassium:	4.1 - 5.6 m.Eq./L. (up to 6.6 m.Eq./L. in the newborn).
Chloride:	98 - 106 m.Eq/L.
$T.CO_2$:	20 - 25 m.Mols/L.
Blood urea:	Varies with feed:-
	1st year human milk: 15 - 30 mg./100 ml.
	1st year cow's milk: 20 - 45 mg./100 ml.
	Over one year: 20 - 40 mg./100 ml.

It is the author's impression that the normal range for serum or plasma sodium in children is too narrow. Many sodium values from 135 - 138 m.Eq./L. are seen in apparently healthy children. Similarly, values for blood urea show much wider variation with diet than one would appreciate from the above normal range.

Measurement of the response of the adrenal cortex to stimulation by exogenous ACTH will not distinguish between primary adrenal cortical hypofunction and that which results from hypopituitarism. There is as yet no means of detecting circulating ACTH in children with normal or hypopituitary function, therefore one must use an indirect assay measuring end-organ response.

Liddle and his colleagues (1958) described alterations of adrenal steroid patterns in man following the administration of a chemical inhibitor of 11-β hydroxylation in cortical synthesis. These workers proposed a new approach to the evaluation of "pituitary reserve" which may be defined as the capacity of the anterior pituitary to secrete ACTH in amounts greater than those required under ordinary circumstances.

An agent which impairs the synthesis of cortisol (Liddle, Island, Lance and Harris, 1958; Jenkins, Meakins and Nelson,1959; Gold et al., 1960), is 2-methyl-1, 2-bis (3-pyridyl)-propanone (SU 4885 - CIBA, "Metopiron", "Metopirone"). In low concentration it is a relatively selective inhibitor of 11-β hydroxylase. In consequence of this inhibition, the secretion of cortisol is replaced by 11-desoxycortisol (Compound S). Compound S is an ineffective suppressor of ACTH secretion; thus as the blood level of cortisol falls there is a rise in the adrenal cortisol secretion of 11-desoxycortisol, provided the pituitary-adrenal axis is intact. Compound S, and its metabolite "tetra hydro S", are measurable in blood and urine as 17-hydroxycorticoids.

Method of Performing the Metopiron Test:

The test was begun at 10 a.m. Five consecutive 24-hour urine specimens were collected using a few drops of chloroform as preservative. "Metopiron" was administered every four hours for

twenty-four hours on the third day, in a dosage of 11 mg. per kilogram body weight. The drug may cause gastric irritation and rapid absorption may lead to vertigo. Each dose was therefore given in powder form after milk and a biscuit. The unpleasant taste was most suitably masked by mixing "Metopiron" with a flavouring agent - strawberry or raspberry jam proved successful. In view of the theoretical possibility of precipitating adrenal failure, intravenous hydrocortisone hemisuccinate was on hand at the cot-side. In more than one-hundred tests, four children vomited one or two doses which were successfully repeated in three cases, and girl patient number 18 felt unwell after the first dose but recovered within two hours. Blood pressure fell from a base-line value of 98/70 mm. Hg. to 80/60 during the twenty-four hour period of "Metopiron" administration.

Urinary 17-ketosteroids were again determined by the second method of Prout and Snaith (1958), and total 17-hydroxycorticoids by the method of Appleby, Gibson, Norymberski and Stubbs (1955).

Interpretation:

In normal adults (i.e. in those in whom there was no reason to suspect adrenal or pituitary dysfunction), there was a two to fourfold increase in urinary 17-hydroxycorticoids (Liddle, Island, Lance and Harris, 1958; Liddle, Estep, Kendall, Williams and Townes, 1959). The increase was noticeable during the period of

administration of "Metopiron" but was usually greater in the following twenty-four hours. A normal range for children has not yet been published but provisional values show a two to threefold increase in total 17-hydroxycorticoids on the fourth day of the test in children over seven years of age (Clayton, 1962). There is little evidence to suggest that there is any significant difference in response to "Metopiron" in children below the age of seven, in view of the uniformity of response to exogenous ACTH in children from five months to twelve years (Clayton, Edwards and Renwick, 1962).

Arguelles, Chekherdemian and Ottone (1962) found that the level of the total urinary 17-hydroxycorticoids was a more sensitive index of response to "Metopiron" in adults than the urinary 17-ketogenic steroids. In children, it is also true that the total urinary 17-hydroxycorticoids are a more sensitive and reliable index than the 17-ketosteroids (Clayton, 1962).

In spite of the fact that it has been shown conclusively in adults that the administration of "Metopiron" results in the increased urinary excretion of Compound S and Tetra-Hydro S, Buus, Binden and Petersen (1962) have challenged the validity of total urinary 17-hydroxycorticoid determinations on the grounds that hydrocortisone and Compound S are not specifically determined. This argument is weak; provided there are sufficient base-line values, the estimation of total urinary 17-hydroxycorticoids is quite legitimate.

These Danish workers also questioned the effectiveness of the four-hourly dosage of "Metopiron" as in blocking 11-βhydroxylase they measured plasma levels of hydrocortisone and Compound S, a method which may be readily criticised because of the wide variation between individuals, the fact that the blood sample may not be collected at the peak of the response, and that the size of the response may be affected by diurnal variation.

The results for the ACTH tests are given in Tables 19 and 22, and for the electrolytes during the ACTH tests in Tables 18 and 21.

Table 17.

ACTH TESTS

BOYS

Patient	17-ketosteroids mg./24 hours				Total 17-hydroxycorticoids mg./24 hours			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
1	2.3	3.4	3.6	4.1	3.1	14.4	17.0	19.2
2	0.7	1.2	3.4	2.4	5.8	25.2	39.8	20.8
3	1.5	1.8	2.7	6.0	1.5	2.9	16.7	32.9
4	0.4	0.7	0.94	2.5	2.2	11.2	11.3	23.2
7	1.4	1.0	1.0	1.0	1.15	10.0	15.8	17.3
8	2.2	3.1	4.3	4.0	2.4	15.6	23.5	18.2
9	0.9	0.47	0.35	0.36	1.0	2.44	2.0	3.0
10	0.4	0.8	0.7	1.0	1.8	10.7	12.9	15.2
11	0.45	0.9	1.2	1.4	1.8	4.8	6.7	10.0
12	2.5	1.2	3.2	3.6	3.7	6.5	19.0	17.5
13	6.7	11.2	4.8	5.6	12.9	18.7	22.0	32.0
14	0.44	1.0*	1.6	3.5	2.6	6.4*	6.8	13.5
15	1.3	4.3	5.6	4.7	4.5	34.7	36.2	30.5
16	0.2	0.2	0.6	1.0	1.0	2.2	9.3	8.8
17	1.0	1.0	1.0	1.7	4.0	8.4	11.0	18.9
18	0.52	1.2	1.3	1.5	2.3	9.0	12.9	16.0
19	2.9	5.7	7.0	3.6	3.4	17.2	37.8	28.7
20	0.8	1.6	1.4	1.9	1.9	5.5	7.6	12.2
21	0.15	0.2	0.8	1.65	1.13	6.64	12.2	18.5

* Approximately 60 ml. lost.

(Continued)

ACTH TESTS

BOYS

(Continued)

Patient	17-ketosteroids mg./24 hours				Total 17-hydroxycorticoids mg./24 hours			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
22	0.62	1.2	3.6	3.4	3.5	7.3	11.5	26.3
23	1.0	0.5	0.45	2.4	4.3	6.3	15.5	24.2
24	0.8	1.3	0.6	1.1	2.6	14.2	14.1	25.5
25	1.0	2.6	6.8	6.0	4.3	19.3	50.9	53.8
26	0.3	0.24	0.32	0.5	0.45	3.6	5.6	10.3
27	0.8	2.7	1.3	2.5	2.2	12.2	10.4	18.9
28	1.2	2.6	5.0	3.8	4.4	13.5	23.2	18.2
29	0.7	2.0	3.4	3.1	7.5	15.4	41.0	54.7
30	3.1	2.6	5.1	9.7	2.6	12.6	20.1	26.3
31	1.1	1.8	3.5	3.8	2.1	16.8	35.9	26.2
32	0.45	1.7	0.9	0.82	3.1	4.7	10.3	18.9
33	0.2	0.4	0.6	0.9	1.4	6.3	10.2	15.3
34	0.41	1.5	3.6	6.3	1.8	16.9	33.5	38.1
35	1.5	2.4	3.5	-	5.8	8.4	14.6	-
36	0.9	1.8	2.65	4.08	4.5	3.6	20.0	24.8
37	2.66*	3.88	1.0	1.46	1.44*	1.43	10.6	17.6
38	3.1	3.7	7.0	5.1	5.8	24.6	39.2	25.0
39	1.1	0.97	1.9	2.8	2.2	12.3	14.7	21.2
40	2.0	5.2	6.2	8.6	2.7	19.2	26.4	26.9
41	0.82	1.15	1.0	2.49	10.3	12.3	14.9	23.6

* Approximately 180 ml. lost.

Table 18.

PLASMA ELECTROLYTES

BOYS

Patient	Sodium		Potassium		Chloride		$T.CO_2$		Blood urea	
	m.Eq./L.								mg./100 ml.	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	140	137	5.5	3.6	100	101	-	-	-	-
2	140	143	4.4	4.6	106	104	-	-	34	28
3	140	137	5.5	3.6	100	101	-	-	-	-
7	139	145	4.2	3.6	102	109	20	20	33	21
8	138	142	5.1	4.4	103	100	26	32	28	22
10	137	138	4.8	4.4	103	103	16	19	25	17
11	138	138	4.3	3.4	101	101	21	23	31	24
13	140	139	4.1	3.9	96	102	-	23	30	19
14	132	135	5.8*	4.8	97	103	17	21	33	32
15	138	140	4.2	4.2	107	105	21	25	44	30
16	132	-	5.6	-	98	-	18	-	44	-
17	138	143	4.7	3.8	99	108	24	21	34	23
18	140	138	4.8*	3.6	105	106	24	22	12	14
19	142	-	5.4	-	108	-	26	-	29	-
20	139	138	5.6	4.3	103	103	20	23	24	33
21	140	-	4.8	-	109	-	18	-	43	-
22	137	-	3.5	-	101	-	20	-	19	-
23	138	138	3.7	4.4	106	106	21	21	26	25
24	141	-	4.4	-	107	-	20	-	28	-
25	140	143	3.9	3.2	106	106	-	-	22	-
26	134	137	5.1	5.6	106	105	22	27	28	39
28	140	138	4.8	4.2	101	105	24	23	20	59
29	135	138	6.4*	4.8	99	103	-	21	28	34
31	130	143	3.7	4.3	100	105	24	24	24	20
32	138	138	4.5	4.4	103	100	23	24	29	19
33	147	138	4.3	4.6	110	102	19	19	51	19
34	139	143	4.2	3.7	103	104	27	23	39	27
35	135	135	3.8	6.5*	104	97	24	-	33	34
36	135	139	4.3	3.6	103	103	21	25	33	17
37	143	143	5.0	4.2	99	103	23	24	34	28
38	140	143	4.6	5.8	106	107	18	22	45	40
39	143	140	4.1	3.6	100	99	23	21	41	21
40	145	-	5.8	-	103	-	25	-	41	-
41	146	143	4.8	4.2	110	104	18	21	28	24

* Haemolysed.

Table 19.

METOPIRON TESTS

BOYS

Patient	17-ketosteroids mg./24 hours					Total 17-hydroxycorticoids mg./24 hours				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
1	0.84	1.6	0.5	1.4	1.2	4.6	5.0	10.6	11.7	3.6
2	1.6	1.1	1.5	1.4	1.5	0.8	1.4	4.7	5.1	5.5
3*	2.0	1.9	1.5	2.6	3.6	3.2	2.0	10.7	23.3	29.0
7	1.0	1.0	0.7	1.8	0.8	2.3	1.3	3.6	3.2	2.6
8	-	2.3	1.7	4.4	2.1	-	5.9	7.3	6.7	4.3
10	0.3	0.4	0.3	0.07	0.2	1.6	1.9	3.2	1.4	1.1
11	0.25	0.2	0.4	0.12	0.4	1.1	0.6	1.0	1.0	1.4
12	0.8	1.5	1.1	1.4	1.8	5.7	3.3	5.5	9.0	7.9
13	4.6	5.6	5.7	6.2	2.4	5.0	6.0	9.5	15.2	10.0
14	0.13	0.84	0.9	0.7	0.5	1.5	2.1	1.1	1.2	1.1
15	1.5	1.1	1.1	1.6	1.4	5.5	5.9	11.7	14.4	8.7
16	0.41	0.35	0.53	0.34	0.44	0.9	0.09	0.8	0.8	0.9
17	1.1	1.1	1.5	0.8	1.2	3.5	5.0	3.5	8.0	4.4
18	0.6	0.76	1.1	0.34	1.25	3.3	5.1	3.6	4.4	4.4
19	2.6	3.9	4.2	2.4	2.9	4.0	6.3	10.5	6.7	5.2
20	0.6	1.4	2.5	1.2	0.7	1.9	3.8	4.3	4.6	5.2
21	0.32	0.22	0.25	0.2	0.4	0.62	0.52	0.44	2.0	0.93

(Continued)

* Metopiron given on 3rd, 4th and 5th days.

METOPIRON TESTS

BOYS

(Continued)

Patient	17-ketosteroids mg./24 hours					Total 17-hydroxycorticoids mg./24 hours				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
22	-	2.0 (48 hrs.)	1.0	3.6 (27 hrs.)	0.9 (21 hrs.)	-	5.0 (48 hrs.)	3.0	4.0 (27 hrs.)	2.0 (21 hrs.)
23	0.55	1.3	2.5	0.6	0.85	5.1	3.7	5.0	6.0	5.7
24	1.0	0.5	1.3	0.8	1.0	2.5	2.3	3.9	4.0	2.4
25	-	1.4	2.5	2.5	1.6	-	1.2	4.8	4.8	5.9
28	1.9	1.9	1.9	2.59	2.2	4.0	8.6	5.6	16.0	4.0
29	1.2	1.6	1.4	2.5	3.7	9.0	7.1	16.2	46.5	26.0
31	1.3	1.0	0.8	1.1	0.8	3.9	1.5	1.5	1.1	1.2
32	0.38	0.92	1.0	0.8	0.4	3.6	3.0	7.3	5.8	1.7
33	0.3	0.4	0.3	0.1	0.4	1.0	1.4	1.8	2.1	1.6
34	1.0	0.91	0.55	1.4	0.49	0.93	0.73	5.2	11.5	2.5
35	0.9	0.8	0.8	1.9	0.57	3.9	4.2	3.4	5.2	4.9
36	0.44	0.7	0.97	0.56	0.51	3.8	3.27	1.08	4.0	1.86
37	0.5	0.32	0.16	0.32	0.43	1.24	0.95	1.12	1.16	1.1
38	3.9	2.1	4.7	3.4	2.2	5.4	5.9	8.6	7.5	6.0
39	1.35	0.9	2.1	1.3	1.4	3.7	2.1	5.2	9.3	2.8
40	3.6	2.2	3.1	2.7	2.6	3.8	8.4	13.5	33.1	8.3
41	0.81	1.5	2.3	2.8	2.1	8.4	7.5	12.8	8.4	7.6

Table 20.

ACTH TESTS

GIRLS

Patient	17-ketosteroids mg./24 hours				Total 17-hydroxycorticoids mg./24 hours			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
3	0.7	2.5	1.95	1.43	2.0	9.5	16.0	14.6
4	0.6	0.9*	1.1	1.5**	2.7	6.1*	10.4	11.3**
5	2.0	2.6	2.2	3.4	0.97	11.4	15.4	14.7
6	0.5	1.2	2.7	2.3	1.8	9.1	22.2	23.0
7	0.36	0.66	0.81	1.4	1.1	1.2	2.0	10.8
8	0.5	0.7	2.1	2.1	4.6	12.9	26.2	40.0
9	0.3	0.35	0.68	1.7	3.6	3.5	8.2	23.0
10	0.31	0.34	0.54	1.5	1.38	2.0	7.6	14.6
11	1.6	2.2	3.0	3.9	6.5	12.6	24.7	37.9
12	0.45	1.2	1.7	3.1	1.3	8.3	20.8	34.6
13	2.2	5.2	5.3	6.1	8.1	43.4	32.8	42.0
14	0.16	0.46	1.1	1.75	0.43	2.25	4.0	11.8
15	0.69	1.3	1.26	3.4	2.4	10.3	16.0	28.8
16	1.5	1.9	3.4	2.6	3.6	11.5	11.0	18.1
17	0.4	1.3	2.5	2.6	2.6	9.7	16.2	33.4
18	1.6	2.6	3.7	4.6	5.6	18.1	30.1	49.8
19	0.48	0.74	1.0	1.12	3.0	14.2	16.5	9.1
20	0.22	0.28	1.9	2.1	0.81	2.8	7.9	18.0
21	3.3	5.1	5.4	7.4	10.3	20.2	24.6	28.6
22	2.1	3.0	4.0	5.2	4.8	27.6	25.2	29.3
23	0.21	0.52	0.96	1.0	0.9	3.66	5.5	9.0
24	0.6	2.3	1.4	3.4	4.8	21.5	17.2	20.1
25	0.4	0.6	0.2	0.6	4.3	5.9	10.9	15.0
26	0.86	4.9	3.9	4.1	13.8	23.7	40.7	18.5
27	3.3	5.4	6.1	5.8	4.5	11.2	13.2	13.0
28	1.0	1.8	1.4	3.0	3.4	8.3	9.5	14.1
29	0.33	0.52	0.72	0.26	1.5	4.45	5.25	3.85

* 56 ml. missing.

** 35 ml. missing.

Table 21.

PLASMA ELECTROLYTES

GIRLS

Patient	Sodium		Potassium		Chloride		$T.CO_2$		Blood urea	
	(m.Eq./L.)								(mg./100 ml.)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3	139	143	6.0*	4.2	104	103	22	22	20	16
5	140	154	3.6	4.4	107	110	24	25	21	15
6	137	138	4.3	4.7	108	105	23	21	20	19
8	140	140	4.6	4.0	107	106	23	22	34	23
10	138	146	4.7	4.6	106	110	21	24	27	19
11	135	138	4.4	4.0	101	99	20	22	22	18
12	141	143	3.8	3.4	104	107	22	33	31	28
13	140	138	5.0	3.6	109	104	-	-	16	-
14	142	143	5.1	4.4	101	107	21	22	27	27
15	136	139	4.5	4.3	102	102	22	21	51	33
16	142	143	4.8	4.1	106	105	22	24	23	24
17	136	142	4.6	3.5	107	104	22	23	34	21
18	134	135	3.8	3.8	103	104	23	18	28	30
19	138	138	4.8	5.1	101	103	22	23	44	24
20	138	150	4.1	5.0	105	110	29	26	46	30
21	139	143	4.3	3.8	102	105	25	24	27	22
23	140	144	4.7	4.3	102	102	-	27	34	27
24	138	138	4.9	3.6	105	104	25	25	21	21
25	140	140	5.9	5.1	102	103	18	21	22	24
26	142	143	3.9	3.3	102	104	16	27	21	18
27	139	142	4.9*	4.0	99	103	21	24	20	15
28	138	138	4.4	3.7	106	108	27	25	26	29
29	135	135	4.8	5.4	98	98	21	22	37	33

* Haemolysed.

Table 22.

METOPIRON TESTS

GIRLS

Patient	17-ketosteroids mg./24 hours					Total 17-hydroxycorticoids mg./24 hours				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
3	0.59	0.61	0.71	1.16	-	2.3	2.0	1.96	4.0	-
4	1.2	0.9	0.9	0.7	0.7	2.7	2.3	1.1	4.5	2.4
5	1.5	1.3	2.1	2.7	3.4	0.81	0.86	0.55	1.1	1.0
6	0.8	0.5	0.6*	0.6	0.5	1.7	1.5	3.3*	6.8	2.5
7	-	0.29	0.17	-	-	-	1.26	0.97	-	-
8	0.5	0.5	0.6	0.9	0.9	3.4	4.6	5.5	18.5	6.1
9	0.39	0.61	0.24	0.52	0.31	3.9	2.9	1.3	-	1.0
10	1.4	0.43	0.46	0.52	0.17	1.6	1.32	1.9	3.1	1.3
11	1.6	2.0	1.4	2.7	3.2	8.1	5.5	9.5	9.6	8.1
13	3.5	3.0	2.0	2.0	3.0	11.8	12.7	9.9	9.2	14.0
14	0.63	0.6	0.42	0.48	0.31	3.4	1.9	0.54	1.14	0.43
15	0.4	0.98	1.2	1.4	0.54	2.2	3.1	2.9	4.7	2.4
16	1.8	1.9	1.0**	1.7	0.9	3.2	4.2	3.6**	4.2	2.5
17	0.82	0.38	0.57	0.34	0.32	1.3	1.05	2.8	2.64	2.36
18	0.5	0.7	1.3	2.0	1.3	4.6	4.7	7.6	13.3	8.6
19	0.5	0.3	0.7	1.1	0.4	1.8	1.8	5.2	2.4	2.5
20	0.7	0.7	0.6	0.2	0.6***	1.3	1.1	1.1	0.8	0.8***
21	2.4	2.3	3.0	2.4	2.9	6.7	4.0	7.7	8.6	4.1
22	2.8	1.8	2.6	4.4	2.9	6.5	5.5	9.1	11.1	5.5
23	0.55	0.78	0.43	0.33	0.23	1.26	0.92	1.52	2.32	1.0
24	-	0.8	1.3	1.4	0.5	-	3.0	7.1	9.7	4.5
25	0.2	0.3	0.4	0.5	0.2	0.6	0.7	1.8	2.2	0.6
27	3.2	5.4	2.7	5.6	2.1	3.0	4.1	5.1	10.4	3.2
28	1.4	1.5	1.0	0.5	1.0	3.1	8.2	7.2	10.2	4.7
29	0.04	0.07	0.13	0.2	0.24	0.48	0.97	1.16	1.79	0.3

* Approximately 90 ml. lost.

** Approximately 45 ml. lost.

*** Unknown volume lost.

SERUM PROTEIN-BOUND IODINE

The determination of the amount of ^{127}I which is bound to serum protein is generally accepted as an index of the amount of circulating thyroid hormone provided that iodine compounds have not been recently ingested, applied to the skin, or employed in contrast media for radiological purposes (e.g. Danowski et al., 1950; Start et al., 1950).

Wayne (1960) confirmed Fraser's view (1956) that the test is of diagnostic value in adult patients with suspected mild hypothyroidism. According to Wilkins (1957) and Andersen (1961) the estimation of the serum protein-bound iodine (P.B.I.), or preferably the butanol-extractable iodine (B.E.I.) is the best single criterion of thyroid function which is currently available in paediatric practice.

Many workers (e.g. de Mowbray and Tickner, 1952; Macgregor and Wayne, 1958; and Wayne, 1960) have experienced serious technical difficulty with several methods but this is chiefly due to the problem of contamination. Although Macgregor and Farrell (1958) estimated the P.B.^{127}I. in eighty-two hypothyroid patients and found that only nine had values greater than 3.0 μg./100 ml., the normal accepted range is usually taken as 3.5 - 7.0 μg./100 ml. (Fraser, 1956). The limits vary slightly with the methods employed and with the laboratory which performs the test, thus the

normal range for P.B.I. determination reported in this study, using the method of Grossmann and Grossmann (1955), is 3.3 - 7.4 µg./100 ml. (Willcox, 1962). There is no sharp delineation between normal and abnormal values and the significance of this will be discussed later.

Studies of serum protein-bound iodine in adults are not applicable to children. In 1925, Cooper reviewed the histological appearance of the thyroid gland at various ages and she interpreted her findings in childhood and adolescence as being compatible with hyperactivity. Evidence has been presented that the "thyroid secretion rate" is greater in young animals than in adults of the same species (Hurst and Turner, 1947; Maqsood, 1950; Wilansky, Newshaw and Hoffman, 1955).

Danowski et al. (1951) have shown that within twelve hours of birth the level of serum protein-bound iodine in the infant is similar to that of the mother during pregnancy or within a few hours of delivery. During the first week of life there occurs a transient but statistically significant increase in the mean value of the iodine fraction. From the sixth to the twelfth week the level of the protein-bound iodine falls below that of the neonate but, until the age of one year, the average value remains higher than that in older children and in the euthyroid, non-pregnant adult.

Danowski et al. (1952) also found lower concentration of

protein-bound iodine in early adolescence in the diabetic and non-diabetic subject. This confirmed the work of Shock (1944) but was not consistent with the widely accepted concept of "physiological hyperthyroidism" at puberty. Since then Oliner et al. (1957) have established normal standards in children for ^{131}I uptake and P.B.^{131}I. They concluded that the thyroid gland in children up to the age of four years and possibly later, is normally in a state of hyperactivity when compared with the euthyroid adult.

Blood samples for P.B.I. determination may be withdrawn at any time of day and the level is unaffected by relation to meals, fever, physical activity or any other variable which distorts the basal metabolic rate (Wolman, 1957). In healthy young adults, Danowski et al. (1949) stated that the level of the protein-bound iodine appeared relatively constant over a period of weeks.

All the determinations in this series were carried out in duplicate on venous blood. Those tests which were abnormal were repeated on another day, and patients with a low value on two separate occasions were investigated using ^{132}I, where this was practicable.

The results of the serum protein-bound iodine estimations are given in Tables 23 and 24.

Table 23.

SERUM PROTEIN-BOUND IODINE

BOYS

Patient	Serum protein-bound iodine µg./100 ml.
2	Greater than 10
3	5.8
5	3.6
7	5.1
8	3.0
9	3.7
10	6.5
11	4.3
12	4.3
13	6.9
15	4.6
16	6.7
17	6.7
18	6.1
19	2.3
20	3.9
21	4.3
22	3.6
23	5.4
24	4.5
25	6.9
26	5.3
28	4.2
29	1.0
30	5.3
31	6.1
33	7.2
34	3.6
35	6.3
36	4.4
37	6.2
38	6.8
39	0.9
40	2.4
41	4.8

Table 24.

SERUM PROTEIN-BOUND IODINE

GIRLS

Patient	Serum protein-bound iodine µg./100 ml.
2	5.9
3	5.4
4	7.0
5	4.3
6	5.8
7	3.2
8	7.0
10	5.6
11	4.1
12	5.0
14	4.5
15	4.6
16	6.6
17	5.2
18	2.5
19	5.4
20	4.2
21	5.8
22	5.7
23	5.0
24	5.4
25	5.5
27	6.7
28	7.2

BASAL METABOLIC RATE

The determination of the Basal Metabolic Rate (B.M.R.), once widely relied upon as the principal test of thyroid function, is now seldom used by some clinics in the diagnosis of hypothyroidism (Wilkins, 1957; Andersen, 1961). However, according to Heald (1962), the B.M.R. is still used with surprising frequency, although more precise methods of assessing thyroid function have been available during the past fifteen years. The Basal Metabolic Rate is not a direct measure of thyroid activity, therefore the results of such investigations must be interpreted with caution.

From the stand-point of diagnosis in the adult, Hortling and Hiisi-Brummer (1959) found determination of B.M.R. and serum protein-bound iodine of almost equal value in 535 patients. Again, in the adult, using the reference standards of Robertson and Reid (1952), Wayne (1960) found that the B.M.R. agreed with the final diagnosis in 77% of cases of hypothyroidism.

Reports in the paediatric literature are not so encouraging, mainly because the determination of B.M.R. in children is complicated by factors not encountered in adults. Technical difficulties frequently arise from the failure to attain satisfactory basal conditions and the selection of the most physiological standard remains controversial (e.g. Talbot, 1936; Talbot, Stewart and Broughton, 1938; Kleiber, 1947; Eichorn,

1955). Behrendt (1949), in a most competent discussion of Basal Metabolic Rate, stated "that for the success and reliability of the B.M.R. test in children over six, the ability to handle children is of far greater importance than technical skill."

Performance of Test:

The B.M.R. was determined by the method described by Behrendt (1949), using the British "Benedict" Portable Metabolism Machine. Some children were allowed their usual diet up to fourteen hours before the test, and thereafter they were fasted until completion of the investigation; others were given a light breakfast of cornflakes and milk at 6 a.m. at the discretion of the Ward Sister. In our experience this ensured more basal conditions, particularly in young children who were apt to become resentful and restless if deprived of a meal in the presence of others. B.M.R. determinations, with and without this light breakfast, showed no significant difference in the same child (Clayton, 1962). The age, height and weight of the patient were noted. On the day before the test, it was the practice of the person carrying out the determination to explain the procedure to the child and to make him familiar with the apparatus. All investigations were repeated, usually on successive days.

Calculation:

The barometric pressure and barometer temperature were read

on completion of the trace. A line was drawn through the base of the respiratory tracings, using the longest flat portion. The number of cubic centimetres of oxygen consumed per minute was then calculated. The dry barometric pressure was obtained by subtracting the brass scale correction, and the water vapour pressure at average temperature during the test period, from the barometric reading (tables: Harrison, 1949). Using the dry barometric pressure the oxygen volume was corrected to N.T.P. Where the assumed respiratory quotient is 0.83, the value obtained was converted to Calories per hour using the following formula:-

$$\text{Volume of oxygen consumed per minute at N.T.P. (c.c.)} \times \frac{60}{1{,}000} \times 4.825$$

The child's surface area was then calculated from a nomogram (Dubois, 1936), and "Total Calories per Hour" was corrected to "Total Calories per Hour per Square Metre of Body Surface".

The results are given in Table 25.

Table 25.

BASAL METABOLIC RATE

BOYS

Patient	Calories per Hour per Square Metre Percentage of Normal for:			
	Age	Weight	Height	Surface Area
2	88	85	90	87
3	110	98.5	101	101
13	116	115	119	116
18	124	108	109	109
19	68	64	84	73
39	94	76	82	79
40	72	67	78	74

BASAL METABOLIC RATE

GIRLS

Patient	Calories per Hour per Square Metre Percentage of Normal for:			
	Age	Weight	Height	Surface Area
5	89	76	72	76
18	68	59	72	64
21	106	98	91	95
24	103	88	82	84
27	133	99	110	102

STUDIES OF THYROID UPTAKE OF ^{132}IODINE

The uptake of ^{132}Iodine by the thyroid after the oral administration of 5 μc. was studied at intervals for six and a half hours in certain patients.

When the uptakes were poor, the patients were tested again after three days' treatment with thyrotropic hormone 5 units daily, intramuscularly (Armour Laboratories Ltd.).

The results are given in Table 26.

Table 26.

STUDIES OF THYROID UPTAKE OF ^{132}IODINE

Patient No. 7 (female):

"Neck uptake"	17.5% at 1½ hours.
	25.9% at 3¾ hours.
	15.8% at 6 hours.

Comment: Normal (the six hour uptake is probably inaccurate).

Patient No. 19 (male):

"Neck uptake" before T.S.H.	9.3% at 2¼ hours.
	8.8% at 4 hours.
	6.4% at 6½ hours.
"Neck uptake" after T.S.H.	9.1% at 2 hours.
	8.6% at 4¼ hours.
	6.8% at 6½ hours.

Comment: Primary hypothyroidism.

Patient No. 29 (male):

"Neck uptake"	8.5% at 2 hours.
	7.5% at 4 hours.
	4.9% at 6½ hours.

No response after T.S.H.

Comment: Primary hypothyroidism.

Continued.

(Table 26, continued)

Patient No. 32 (male):

"Neck uptake"	24.1% at 2 hours.
	26.0% at 4 hours.
	34.9% at 6½ hours.

Comment: Normal thyroid uptake.

Patient No. 34 (male):

"Neck uptake" before T.S.H.	14.4% at 3¼ hours.
	15.5% at 6 hours.
"Neck uptake" after T.S.H.	15.4% at 4 hours.
	13.3% at 6½ hours.

Comment: No change after T.S.H. The "neck uptakes" are probably down below the normal range (more than 15-18% at 6 hours) but they are not extremely low.

DISCUSSION

In children, as in adults, the most important aspect of clinical medicine is diagnosis and the pathologist, like the physician, should evaluate his laboratory findings against the background of a carefully taken history and examination. The interpretation of the history in paediatric practice involves some assessment of the informant as a witness (Ellis, 1960) and although the chief complaint is of value, sometimes it can be very misleading.

Thus, in this series of investigations on seventy children, forty-two were brought to hospital because of "small size", or of "small size" in association with some other complaint (e.g. convulsions). However, sixty-four were found to be below the third percentile for height, including two girls whose measurements are not tested but who were well below this rating. In some instances no mention of shortness of stature was made by the informant; of greater importance, small size was not infrequently overlooked by experienced clinicians. The physical examination of the child is incomplete without the determination of height and weight but these values would be of greater significance if plotted on standard charts (e.g. Tanner, 1958), particularly on those patients who report regularly to hospital.

BIRTH WEIGHT

BOYS

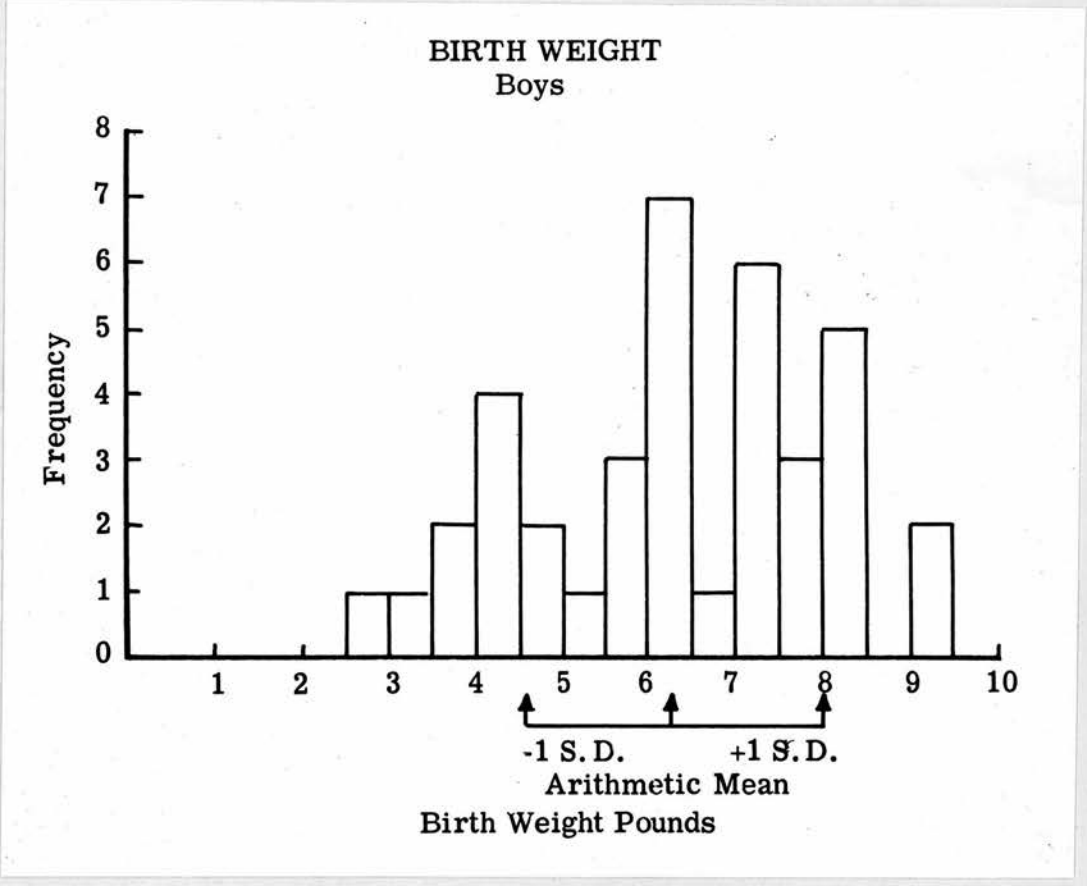

Fig. 2.

BIRTH WEIGHT

GIRLS

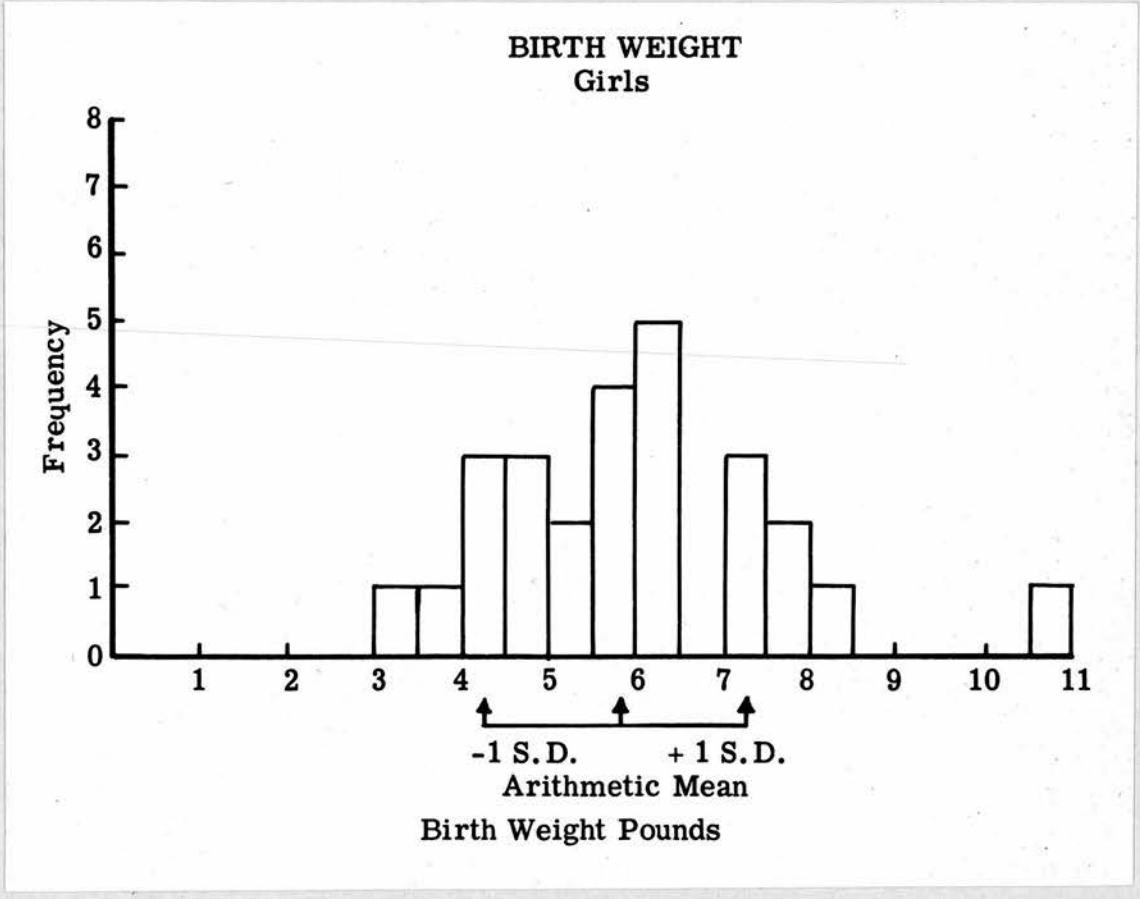

Fig. 3.

Pregnancy and Confinement.

There was no significant relationship between the incidence of abnormal pregnancy and confinement and dwarfism.

Birth Weight.

The birth weights of boys and girls are shown on the accompanying histograms.* There is a suggestion of two distinct groups in both sexes. The mean birth weight for boys was 6 lb. 4 oz. ± S.D. 1 lb. 11 oz., and that for girls was 5 lb. 13½ oz. ± S.D. 1 lb. 10 oz. These are significantly lower than the normal values obtained in the Oxford Survey (Lancet, April, 1955), where the mean birth weight for 298 boys was 7.63 lb. ± S.D. 1.08 lb., and that for 282 girls was 7.31 lb. ± S.D. 1.06 lb.

Although most of the children in this study were born in the South-Eastern area of England, the Oxford values were chosen for comparison in preference to those of Falkner for London children (1958). Falkner did not include standard deviations in his paper, but his mean values of 7.6 lb. for boys and 7.1 lb. for girls show a good correlation with those of the Oxford Survey.

Birth Order and Maternal Age.

The analysis of birth order and maternal age by comparison

* Figs. 2 and 3.

with non-affected siblings is only satisfactory where the family is complete (Carter, 1962), and this analysis could not be undertaken because most of the families in this study are incomplete. The alternative method of testing maternal age and birth order effects, which makes use of all the index cases, is to compare maternal age and birth order of each index case with that of births in the same year in the general population. Accurate details of live and still-births could not be obtained, therefore this method of analysis was not attempted.

Sex Ratio.

Of seventy children studied, thirty-six boys and twenty-six girls were on or below the third percentile for height against chronological age. This gave a sex ratio of 0.72.

Consanguinity.

None of the parents were blood relatives.

Twins.

There was one index case with twins (girl no. 22). She was the smaller and only survivor of the twin pair.

Triplets.

Boy no. 35 was the smallest and sole survivor of triplets.

Parental Heights.

The parental heights of the children studied were as follows:-

Boys: Maternal height 63.3 inches ± S.D. 2.7 inches.
Paternal height 67.7 inches ± S.D. 3.8 inches.

Girls: Maternal height 61.6 inches ± S.D. 2.1 inches.
Paternal height 66.8 inches ± S.D. 3.45 inches.

Parental heights were compared with values published by Tanner, Healy, Lockhart, MacKenzie and Whitehouse (1956). These were:-

Males: 67.5 inches ± S.D. 2.56 inches.
Females: 63.2 inches ± S.D. 2.24 inches.

For the purpose of comparison these values were corrected from millimetres to inches and corrected from supine to standing height by means of Palmer's Tables (1932). They agree closely with the unpublished data of Tanner and Whitehouse (1962):-

Males 68 inches, females 63 inches.

No significant difference was apparent between those figures for the children under study and normal values.

Age at which "Small Size" was First Noted.

At birth, prospective dwarfs are indistinguishable from other newborn infants in the population at large. Growth continues normally throughout infancy to above two to three years (Martin and Wilkins, 1958), and Seckel (1960) has stated that dwarf growth manifests itself between one and three years or later, but these statements are not confirmed by the present study. Twenty out of forty-one boys and sixteen out of twenty-nine girls were found to be of small size or showed slowing of growth within the first year of life.

In eighteen of the seventy children studied the final diagnosis was as follows:-

Boys: Patient	Final Diagnosis
2	Cyst of Rathke's Pouch.
8	Erythrogenesis imperfecta; transfusion haemosiderosis.
13	Absent testes.
15	Arrested hydrocephalus.

Boys (continued):

Patient	Final Diagnosis
17	Evidence of hypopituitarism; at thirtieth percentile for height against chronological age.
20	Chronic regional ileitis.
25	Radiological absence of corpus callosum.
29	Primary hypothyroidism.
30	Craniopharyngioma.
32	Progeria.
34	Primary hypothyroidism.
37	Eczema.
38	Diabetes insipidus.
40	Primary hypothyroidism.

Girls: Patient	Final Diagnosis.
1	Mongol, coeliac disease.
13	Coeliac disease.
18	Primary hypothyroidism.
26	Turner's syndrome.

Of the remainder who had a complete endocrine investigation, the following groups emerge:-

BOYS

Group A: Patient no. 3.

Normal thyroid function, normal adrenocortical response to ACTH, good response to "Metopiron", normal insulin tolerance.

Group B: Patients 7, 9, 10, 11, 12, 16, 18, 33.

Normal thyroid function, subnormal adrenocortical response to ACTH, impaired or absent response to "Metopiron", abnormal insulin tolerance.

Group C: Patient no. 21.

Normal thyroid function, subnormal adrenocortical response to ACTH, good response to "Metopiron", abnormal insulin tolerance.

Group D: Patients 23, 24, 31, 28, 36, 41.

Normal thyroid function, normal adrenocortical response to ACTH, impaired or absent response to "Metopiron", abnormal insulin tolerance.

Group E: Patient no. 19.

Impaired thyroid function, normal adrenocortical response to ACTH, impaired response to "Metopiron", abnormal insulin tolerance.

GIRLS

Group W: Patients 6, 8, 17.
Normal thyroid function, normal adrenocortical response to ACTH, good response to "Metopiron", abnormal insulin tolerance.

Group X: Patients 4, 5, 14, 16, 20, 23, 28.
Normal thyroid function, subnormal response to ACTH, impaired or absent response to "Metopiron", abnormal insulin tolerance.

Group Y: Patients 10, 19, 25, 27.
Normal thyroid function, subnormal adrenocortical response to ACTH, good response to "Metopiron", abnormal insulin tolerance.

Group Z: Patients 15, 21, 22.
Normal thyroid function, normal adrenocortical response to ACTH, impaired or absent response to "Metopiron", abnormal insulin tolerance.

Each group was examined in relation to certain physical attributes, as follows:-

BIRTH WEIGHT, BOYS (OUNCES).

Group	Mean	6
A	112	0
B	95.3	21
C	112	0
D	113	14

COMPARISON WITHIN GROUPS

Group	"P"
BD	0.093

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUES

(Where N = normal value)

Group	"P"
NA	0.5 - 0.6
NB	< 0.01*
NC	0.5 - 0.6
ND	0.1 - 0.2

BIRTH WEIGHT, GIRLS (OUNCES).

Group	Mean	σ
W	104	16
X	92.5	27
Y	73	19
Z	66	13

COMPARISON WITHIN GROUPS

Group	"P"
WY	0.075
WZ	0.075

* Indicates "P" values of possible significance.

$<$ = less than.

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUES

(Where N = normal value)

Group	"p"
NW	0.1 - 0.2
NX	< 0.01*
NY	< 0.01*
NZ	< 0.01*

MATERNAL HEIGHT (INCHES)

BOYS

Group	Mean	σ
A	66.0	0
B	63.0	2.0
C	67.0	0
D	63.0	2.7
E	60.0	0

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUE

(Where N = normal value)

Group	"p"
NA	0.1 - 0.2
NB	> 0.9
NC	0.05 - 0.1
ND	> 0.9
NE	0.1 - 0.2

* Indicates "P" values of possible significance.

> = greater than.

< = less than.

MATERNAL HEIGHTS (INCHES)

GIRLS

Group	Mean	σ
W	62.2	0.8
X	62.5	1.3
Y	60.0	1.3
Z	64.0	0

COMPARISON WITHIN GROUPS

Group	"P"
WX	0.051
YZ	0.075
XY	0.02 - 0.05*

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUE

(Where N = normal value)

Group	"P"
NW	0.4 - 0.5
NX	0.5 - 0.6
NY	$<$ 0.01*
NZ	0.7 - 0.8

* Indicates "P" values of possible significance.

$<$ = less than.

PATERNAL HEIGHT (INCHES)

BOYS

Group	Mean	σ
A	67.0	0
B	68.7	3.3
C	69.0	0
D	66.8	4.0

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUES

(Where N = normal value)

Group	"P"
NA	0.8 - 0.9
NB	0.2 - 0.3
NC	0.5 - 0.6
ND	0.5 - 0.6

PATERNAL HEIGHT (INCHES)

GIRLS

Group	Mean	σ
W	63.3	0.6
X	65.1	1.8
Y	62.5	4.8
Z	72.0	0

COMPARISON WITHIN GROUPS

Group	"P"
WZ	< 0.01*
XZ	0.02 - 0.05*

(Only one value in Group Z)

COMPARISON OF MEAN GROUP VALUE WITH NORMAL VALUE

(Where N = normal value)

Group	"P"
NW	< 0.01*
NX	0.05 - 0.1
NY	> 0.9
NZ	0.05 - 0.1

(Only one value in Group Z)

Height Percentile Rating for Skeletal Age.

No significant difference was found on examining the percentile rating for height against skeletal age between groups.

All standard deviations and probabilities are based on the assumption that there is a symmetrical distribution, i.e. no skew.

* Indicates "P" values of possible significance.

$<$ = less than. $>$ = greater than.

All standard deviations are ± the value given.

Only "P" values around 0.05 are tabulated. A "P" value of below 0.05 suggests a significant difference between two groups but the numbers involved in this study are so small that no conclusive results can be reached.

Of seventy children studied, eighteen showed evidence of a primary disease process initially correlated to the anterior pituitary gland. The endocrine status of thirty-four of the remainder was evaluated as completely as possible, and five of these children (boy no. 21; girls 10, 19, 25 and 27) showed varying degrees of primary adrenal insufficiency. Twenty-nine children had biochemical evidence of hypopituitarism and none of these showed signs of a destructive lesion. However, it is possible that symptoms and signs of a specific process may become apparent at a later date.

A number of instances of dwarfism have been reported in which there was no suggestion of a destructive lesion of the pituitary gland and these have been referred to as dwarfs of the Lorain-Levy type, in contradistinction to the Primordial Dwarfs. It has already been stated in the introduction to this thesis that such classifications on purely clinical grounds may be incorrect and potentially dangerous. For example, half of the thirty-four children under discussion showed varying degrees of

adrenal insufficiency, and two boys (nos. 9 and 16) showed no response to a standard ACTH test on the absence of clinical signs. Another boy (no. 26) collapsed and died during a lumbar air-encephalogram; he had shown a very poor response to ACTH and biochemical evidence of hypopituitarism was confirmed at autopsy.

It is possible that hypopituitarism may exist in the absence of a destructive process affecting the anterior lobe, and that a condition may exist in the human which is comparable to that in the dwarf silver mouse (Smith and MacDowell, 1930, 1931). This condition in mice is attributed to the hereditory absence of eosinophil cells in the hypophysis and a few autopsy reports on pituitary dwarfs support this possibility (Kraus, 1926).

Single hormonal deficiencies of the anterior pituitary have been described in children (Cleveland, Green and Migeon, 1960) but such claims are scarcely valid in the absence of a sensitive assay for Human Growth Hormone, and in the absence of further support of Fitschen's claim (1962) to have detected urinary gonadotrophin activity in apparently normal children.

It is tempting to attribute the findings of dwarfism, retarded skeletal age and abnormal insulin tolerance in girls 6, 8 and 17 to a pure Growth Hormone deficiency, but boy no. 3 showed no biochemical abnormality and yet he was dwarfed and his skeletal age was considerably retarded. The findings in this boy suggest deficiency of a "bone-age" hormone perhaps related to

Growth Hormone, as MSH is related to ACTH. Further chemical studies on the Growth Hormone molecule may confirm this possibility in view of the isolation of an active small molecular weight compound from the larger ACTH molecule, and the separation of an insulin-like factor from Growth Hormone (Ottaway and Paul, 1957; Huggins and Ottaway, 1960).

Boy no. 22 and girl no. 11 showed no biochemical evidence of hypothyroidism on determination of basal metabolic rate and serum protein-bound iodine, but there were clinical signs of hypothyroidism in both children. ^{132}I could not be carried out. Thyroid function often diminishes more slowly in hypopituitarism than in primary thyroid disease (Wayne, 1960). It is, therefore, not surprising that these tests have given inconclusive results.

In boy no. 19 ^{132}I studies were consistent with a diagnosis of primary hypothyroidism but these were at variance with the clinical findings, particularly as this child was very alert mentally. In addition, he did not show the large increase in urinary total 17-hydroxycorticoids after "Metopiron" which was evident in those children with an unequivocal diagnosis of primary hypothyroidism (girl no. 18; boys 29, 34 and 40). The reason for this is not known.

PROVISIONAL CLASSIFICATION OF HYPOPITUITARY DWARFISM IN PRE-PUBERTAL CHILDREN

The diagnosis of hypopituitary dwarfism in pre-pubertal children is difficult and this has been stressed by many authors including Martin and Wilkins (1958) and Wilkins (1957), but the findings in this thesis indicate that treatment cannot be delayed until a conclusive diagnosis is established after puberty. This statement is particularly applicable to those children who present with adrenal insufficiency where the defect may be remediable by the administration of ACTH or cortisone.

The increasing availability of Human Growth Hormone provides a non-androgenic anabolic agent of established potency. Although no conclusive results for long-term therapy are available in the four boys studied (nos. 12, 24, 31 and 33), preliminary findings suggest that this hormone effects an increase in height and muscle mass when given in adequate dosage (e.g. 25 mg. by intramuscular injections twice weekly).

The choice of therapeutic agent is dependent on the diagnosis and a provisional classification of hypopituitary dwarfism in pre-pubertal children is suggested for research purposes. This is based on the findings in the thirty-seven children who had a complete endocrine investigation using standard tests.

Group I	Normal thyroid function
	Normal adrenocortical response to ACTH
	Normal response to "Metopiron"
	Normal insulin tolerance
	Retarded skeletal age
Group II	Normal thyroid function
	Normal adrenocortical response to ACTH
	Normal response to "Metopiron"
	Abnormal insulin tolerance
	Retarded skeletal age
Group III	Normal thyroid function
	Impaired adrenocortical response to ACTH
	Impaired response to "Metopiron"
	Abnormal insulin tolerance
	Retarded skeletal age
Group IV	Normal thyroid function
	Impaired adrenocortical response to ACTH
	Normal response to "Metopiron"
	Abnormal insulin tolerance
	Retarded skeletal age

Group V — Normal thyroid function
Normal adrenocortical response to ACTH
Impaired response to "Metopiron"
Abnormal insulin tolerance
Retarded skeletal age

Group VI — Abnormal thyroid function
Normal adrenocortical response to ACTH
Impaired response to "Metopiron"
Abnormal insulin tolerance
Retarded skeletal age

It is plain that this attempt at classification is incomplete because of the small numbers of children involved, and the absence of a sensitive assay for Human Growth Hormone and of detailed studies of urinary gonadotrophin using a sensitive method like that of Fitschen (1962). Consideration of these parameters in diagnosis is therefore precluded.

Group IV would appear to consist of those dwarfs with primary adrenal deficiency, but this condition is not usually associated with dwarfism. It was considered desirable to include this group at this stage, though views on it may change with increasing experience.

HYPOGLYCAEMIA AND HYPOPITUITARISM

The frequency and severity of insulin sensitivity in children of short stature prompted investigation of the occurrence of hypoglycaemic convulsions in these children (Renwick, 1962). Hypopituitarism as a cause of hypoglycaemia is said to be rare, and in their study of twenty-six cases of pituitary dwarfism, Martin and Wilkins (1958) described two patients who had hypoglycaemic episodes in early childhood but in whom the convulsions had resolved spontaneously.

It is the author's experience that hypoglycaemia is frequently not considered in the differential diagnosis of a child with convulsions and the condition is sometimes overlooked. A retrospective diagnosis of hypoglycaemia may be difficult or impossible to establish.

Twelve children in the present study had a history of fits and of these, one was found to have a craniopharyngioma (boy no.30). In ten of the remaining patients no organic cause could be found for their convulsions, but all of them were below the third percentile for height. The last patient was small, but not dwarfed (boy no. 17). Most of the children had fits between the ages of six months and five years, and in some the convulsions appeared to become less frequent or to disappear completely.

Ten children had fits in the morning before breakfast, or at

a time when their appetite was poor, and they were "off their food". Two children, boy no. 21 and girl no. 20, had fasting blood sugars below 50 mg./100 ml; the remainder were in the normal or low normal range. It should be indicated that these determinations were done by the modified method of Folin and Wu (Wilkinson, 1960) and the values are about 10-15 mg./100 ml. higher than true blood glucose estimated by glucose oxidase.

All of the children were markedly sensitive to a "half" dose of insulin on testing and all of them showed a decreased capacity to correct this insulin induced hypoglycaemia. An exception was found in girl no. 28, who was sensitive to a "half-dose" of insulin but who later showed a normal response to a "full" dose.

Eight of the twelve children who had a complete set of endocrine investigations could be grouped as follows:-

Girls 10, 25, 27; and boy 21	Normal thyroid function, abnormal adrenocortical response to ACTH, good response to "Metopiron", abnormal insulin tolerance.
Girls 20, 28; and boy 17	Normal thyroid function, abnormal adrenocortical response to ACTH, impaired or absent response to "Metopiron", abnormal insulin tolerance.

Boy 31	Normal thyroid function, normal adrenocortical response to ACTH, absent response to "Metopiron", abnormal insulin tolerance.

Patient no. 26, a dwarfed boy with a history of hypoglycaemic convulsions could not be fully investigated, but he showed a very poor response to exogenous ACTH during a standard test. This child died suddenly during air-studies and at autopsy the main endocrine findings were pancreatic hypoplasia, and atrophy of the zona reticularis and zona fasciculata. The pituitary was normal on macroscopic examination. Another child (girl no. 7), who was not fully investigated, also had a very poor response to ACTH.

It may be concluded that four children who were under the third percentile for height and who had a history of fits, showed primary adrenal insufficiency (girls nos. 10, 25, and 27; boy no. 21). It is of interest that a sibling of boy no. 21 died during the second stage of labour and at autopsy no adrenal tissue was found.

Two girls (nos. 20 and 28) and one boy (no. 17) were considered to be deficient in ACTH, and boy no. 31 showed no response to "Metopiron" but he had a good response to ACTH. Again,

it should be pointed out that girl no. 28 had a sibling of five and a half weeks who was admitted to another hospital because of feeding difficulty and convulsions.

It was possible that some of these children may have had a deficiency of Growth Hormone in addition to these endocrine abnormalities.

The hypophysectomised human, in common with hypophysectomised animals, is unable to withstand prolonged fasts and is profoundly sensitive to insulin. Boy no. 2 provides an illustration of this, (see Fig. 4).

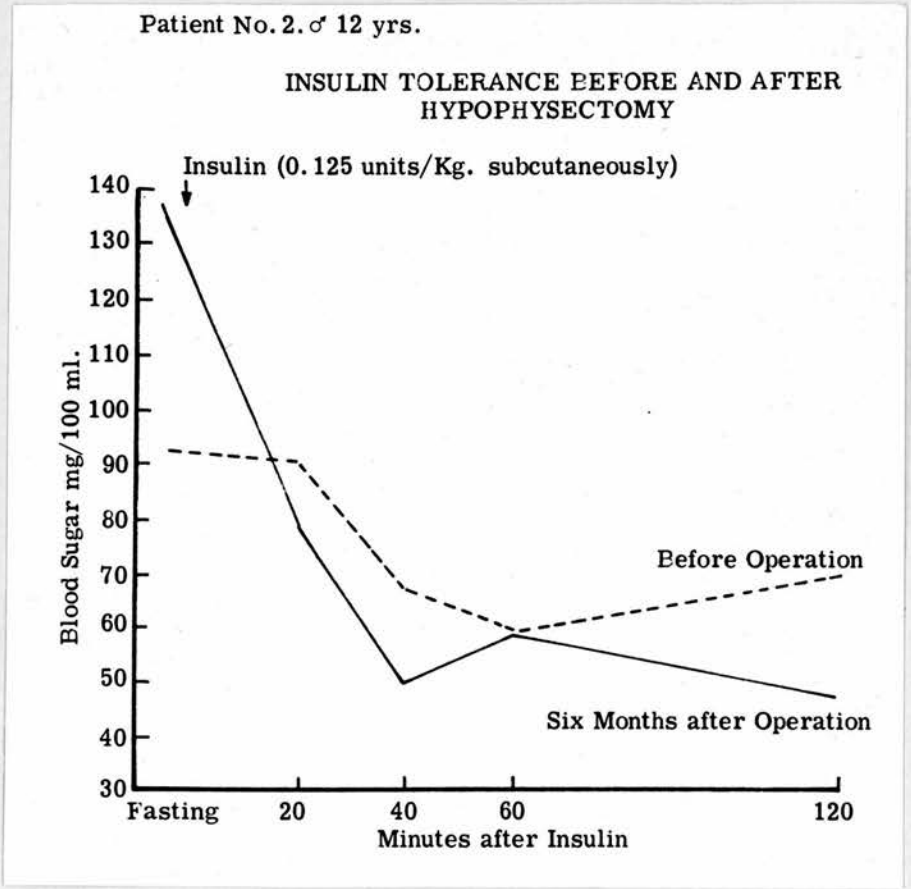

Fig. 4.

An insulin tolerance test before operation revealed a marked sensitivity to insulin and a failure of the blood sugar level to return to fasting values. Six months after hypophysectomy and the removal of a cyst of Rathke's Pouch, the degree of insulin sensitivity and hypoglycaemia unresponsiveness was more striking.

The use of C-14 labelled glucose has increased the understanding of insulin sensitivity in animals and this has been shown to be due to two factors in the hypophysectomised dog (Wall, Steele, De Bodo and Altszuler, 1957; Altszuler, Steele, Dunn, Wall and De Bodo, 1959). These workers found an increase in the rate of removal of plasma glucose in the hypophysectomised dog and this resulted in a fall of plasma glucose below normal levels. The flow of glucose from the liver into the plasma in response to an insulin-induced hypoglycaemia was not increased in the hypophysectomised dog and this resulted in a continuation of the hypoglycaemic state.

The "anti-insulin" effect of Growth Hormone has been demonstrated in numerous experiments but the mechanism of action is unknown. De Bodo and Altszuler (1958) have discussed the question of whether this represents a direct or indirect antagonism between Growth Hormone and insulin.

In the present studies, insulin tolerance tests were carried out before the administration of Human Growth Hormone (M.R.C. Raben preparation) in boys 12, 24 and 33. The striking feature*

* See Figs. 5, 6 and 7.

Patient no. 12. Male. 12 years

EFFECT OF HUMAN GROWTH HORMONE ON

INSULIN SENSITIVITY

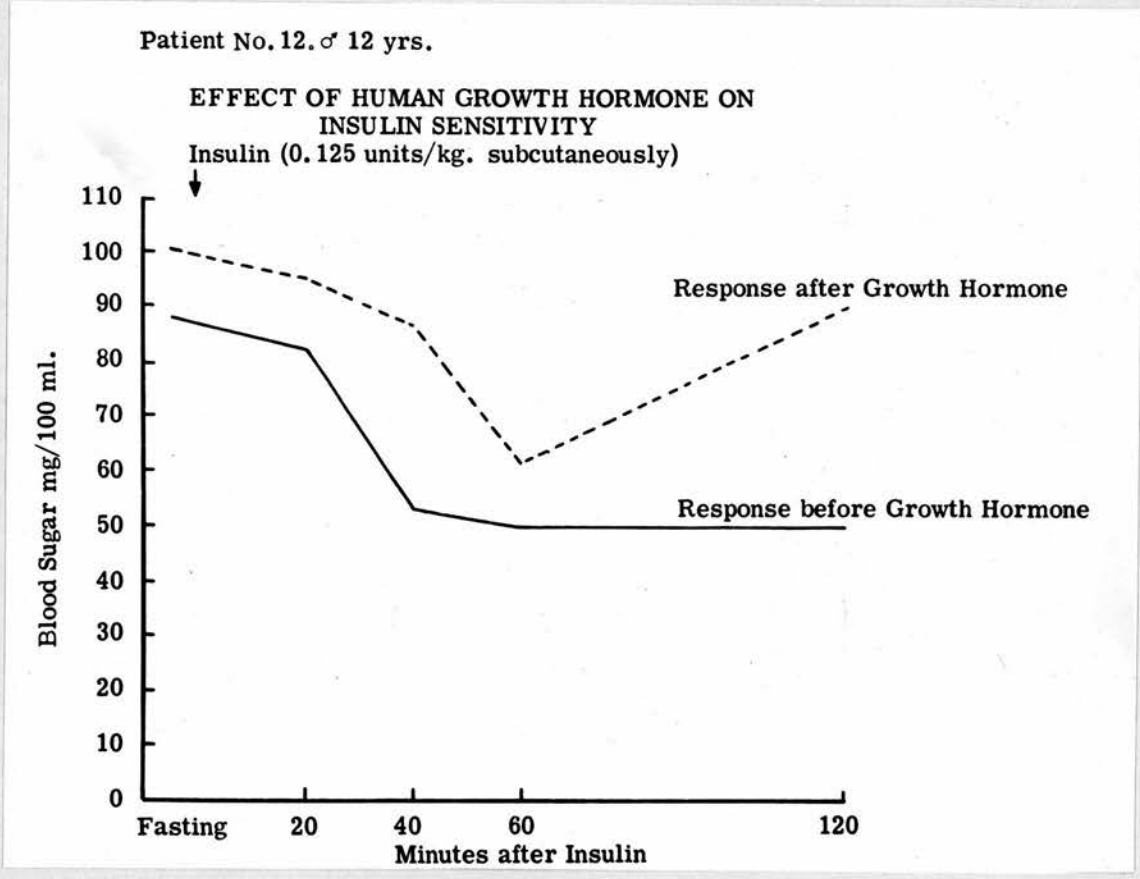

Fig. 5.

Patient no. 24. Male. 11 years

EFFECT OF HUMAN GROWTH HORMONE ON

INSULIN SENSITIVITY

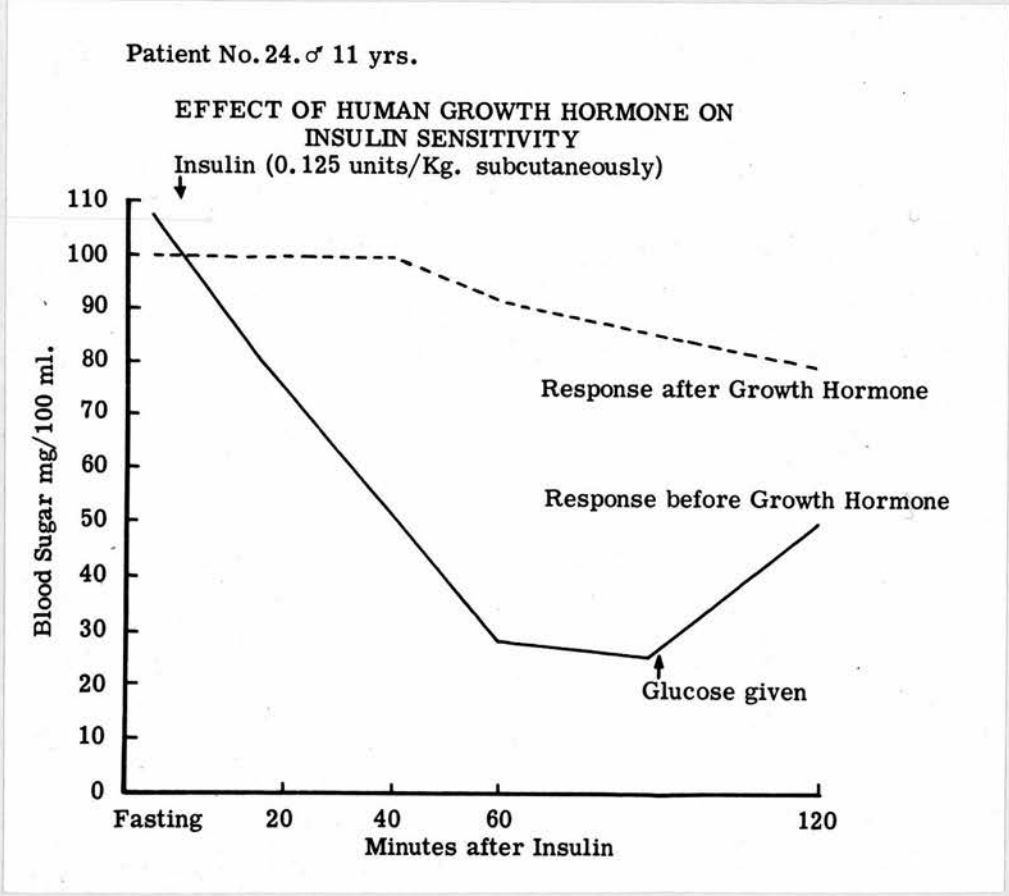

Fig. 6.

Patient no. 33. Male. 6 years

EFFECT OF HUMAN GROWTH HORMONE ON INSULIN SENSITIVITY

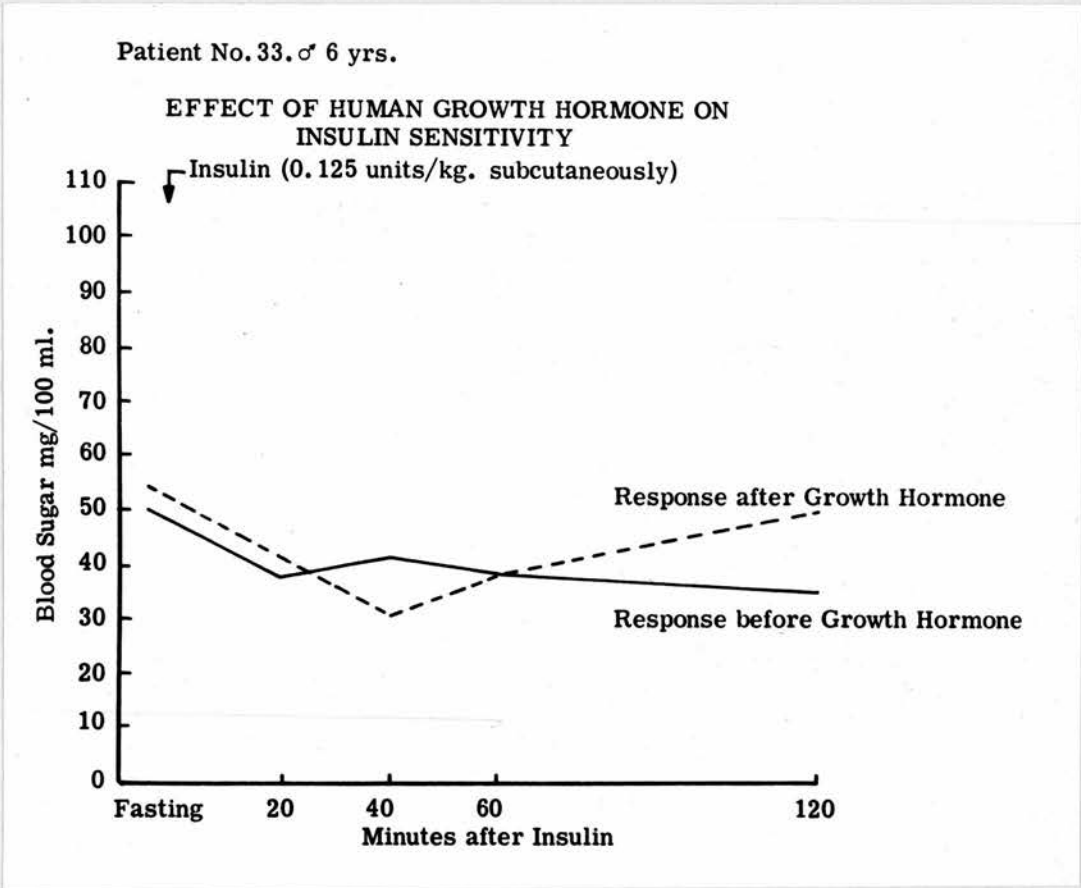

Fig. 4.

was the abolition of sensitivity in patients 12 and 24, but little change was observed in no. 33. The last mentioned child was shown to have a moderate degree of impairment of adrenocortical function and this raised the question of whether the action of growth hormone on carbohydrate metabolism in the human requires an intact adrenal cortex; but the present studies are incomplete and too few to provide strong support for this theory.

It is widely accepted that the anterior pituitary hormones which exert the greatest influence on carbohydrate metabolism are ACTH and Growth Hormone. It is of great significance that ACTH, Growth Hormone and adrenal corticosteroids increase the output of glucose by the liver in the post-absorptive state, and that they also provide a means whereby the liver releases glucose into the blood stream when the need arises. However, the mechanisms involved, particularly those concerned with the actions of Growth Hormone on carbohydrate metabolism, remain uncertain.

DETAILS OF SOME PATIENTS WITH A HISTORY OF HYPOGLYCAEMIA

PATIENT NO. 17:

This six-year old boy was well until two weeks before admission, when he had diarrhoea associated with central abdominal colicky pain. He recovered and returned to school. The following day he passed three large stools and his mother put him to bed. She returned to find him unconscious; there was no warning cry. The child had a fixed stare and his body was twitching. There was no cyanosis. The family doctor gave him soluble phenobarbitone which stopped the "twitchings" for about twenty minutes. The child remained unconscious for three hours and he was incontinent of urine. On admission to hospital the blood sugar level was 12 mg./100 ml. Four hours after intravenous glucose, the blood sugar level was 20 mg./100 ml.

The child had had very little to eat on the day before this episode, and he had no breakfast on that morning. There was a history of a previous fit which occurred at the age of two years, again before breakfast. At that time he was cyanosed and he "jerked" for fifteen minutes.

PATIENT NO. 21:

This boy had "trembling of the limbs" during the first two

weeks of life. He was treated with sedation and the trembling gradually disappeared. At the age of nine months the child began to have convulsions. These usually occurred first thing in the morning when he suddenly cried out, became floppy, and lost consciousness. During a fit his eyes rolled and his legs and arms twitched. The child's mother placed him in a hot bath and the twitching stopped. He would sleep from one to twelve hours after a fit. In spite of sedation with phenobarbitone, the child had an average of two fits a month. He usually did not eat very well before a fit, and the child's mother came to expect an attack whenever he went off his food.

PATIENT NO. 26:

At the age of four months this sixteen-month old boy had one convulsion which was associated with a high temperature. There were no further convulsions until fifteen months of age, when he had another which lasted one hour. At that time "his eyes rolled and he was out but not unconscious". One fit was observed in hospital, and a blood sugar taken during the fit was 44 mg./100 ml. The child made a complete recovery within thirty minutes of the intravenous administration of 10 ml. of 50% glucose.

PATIENT NO. 27:

When this boy was two years old, his mother heard a scream

early one morning and she found the child convulsing and frothing at the mouth. This fit lasted for more than an hour. He was in perfect health before and after the fit.

Eight months later the child was found unconscious, there were no convulsions and he responded to intravenous glucose within two hours. There were no further episodes until the child was four years old, when his mother was unable to rouse him one morning. He was limp, and he remained unconscious on that occasion for three hours. Again he responded to intravenous glucose.

PATIENT NO. 30:

This boy, aged six years and ten months, was well until a few months before admission, when he complained of headaches and fits which were diagnosed as Petit Mal. They were of short duration (five to ten seconds) and sometimes the child had five or six fits a day, with intervals of half to three quarters of an hour between each episode. Usually they occurred first thing in the morning.

During a fit the boy would hold his head still, stare, then drop his head. This was followed by normal behaviour. He was incontinent of urine during a fit on three occasions. Treatment with "Tridione" had no effect.

PATIENT NO. 31:

This boy was well until he was three years old, when he had a fit which lasted twenty minutes. The informant described tonic and clonic spasms and the child was incontinent of urine. Since that time he had many fits without tonic or clonic spasms and without urinary incontinence. The fits usually occurred in the morning, never at school or at play. There was no aura. The child suddenly fell unconscious on rising. He usually got out of bed and dropped to the floor where he lay unconscious for a few minutes, then he went to sleep for three or four hours. He was usually flushed at the start of a fit, then he went mauve around the mouth. The child had a fit about every three to four months.

PATIENT NO. 7:

This two-year old girl had a history of convulsions from the age of one year. "She seemed to go stiff, her eyes rolled, she frothed at the mouth, and she went mauve about the lips". She was unconscious for varying lengths of time (not stated) and she slept after each fit. They seemed to increase in frequency and when the child was seen on admission she had two or three fits a day and sometimes two or three in the night.

PATIENT NO. 10:

At the age of four and a half years this girl had diarrhoea

for one week. There was slime and blood in the stools. No other member of the family was affected. One week after recovery, she got up in the morning feeling cold and drowsy. Just before lunch her eyes rolled up, she frothed at the mouth, and first her arms jerked and then her legs. Her head turned to the left and she was blue around the mouth. She was unconscious for ten minutes. There was no incontinence or fever but the child was off her food all that day. She recovered completely by the following day and she had no further fits.

PATIENT NO. 20:

This ten-year old girl had a history of "nervous spasms" which lasted a few seconds during which time she was unable to speak and she appeared to be terrified. No further details were given, but the child was found to have fasting blood sugars of below 50 mg./100 ml. during a hospital admission.

PATIENT NO. 25:

This child was seen at the age of sixteen months on account of convulsions and failure to grow. At the age of eight months she had a fit in which she stiffened and her eyes rolled back. She then became very limp. There was no associated fever and she had no infections at that time. One month later the child had

three or four fits in one day. Between the ages of nine and eighteen months there were three recorded fits and two of these occurred when the child's appetite was "very, very poor". Treatment with phenobarbitone was ineffective.

PATIENT NO. 27:

This girl was first seen at the age of sixteen years on account of small stature and absence of secondary sex characteristics. As an infant she would not take her feeds well and there was a history of "spells" from the age of seven months to three years. There were about twelve such episodes during that time and they were most severe at one year of age. Unfortunately, this child was referred from Cornwall and it was not possible to obtain a detailed history of these "spells", which were described by her doctor as "breath-holding" attacks.

PATIENT NO. 28:

When this girl was six months of age she went off her feeds, and in the middle of one day she had a fit during which her eyes rolled, her legs curled up, and she was dazed. This fit lasted half an hour and she slept for hours afterwards. At that time she had one fit a day for one month, then they gradually became less frequent. The child was perfectly well between fits, but she

occasionally vomited during a fit. She also lost weight at that time.

At the age of six and three quarter years, the child's mother thought that the patient had tripped and fallen, but she made no attempt to get up and her mother found her lying stiff, with her eyes staring. She was not incontinent. When she came to the child cried hysterically for about half-an-hour. She had a second fit, which was equally severe, the same evening. The local doctor treated her with phenobarbitone and penicillin but she had nine further episodes between that time and her admission to hospital. The fits had become gradually less severe.

There was also a history of headaches and drowsiness at school. The child was off-colour for about a week, but she recovered spontaneously. A younger sibling aged five and a half weeks also had a history of feeding difficulty and convulsions.

HYPONATRAEMIA

Most patients with lesions of the anterior pituitary gland are not "salt-losers" (Luetscher and Axelrad, 1954; MacLean, Lipsett, Ray and Pearson, 1955; Hokfelt, Luft, Ikkos, Olivecrona and Sekkenes, 1959), and this is supported by the plasma sodium values in the present study. Only three girls had plasma sodium values of 135 m.Eq./L. and below. No. 11, who had biochemical evidence of impairment of the pituitary-adrenal axis and who showed some clinical signs of hypothyroidism, had a plasma sodium level of 135 m.Eq./L. Girl no. 18, with unequivocal evidence of primary hypothyroidism, had a plasma sodium value of 134 m.Eq./L. on the first day of the ACTH test. Girl no. 29, who showed no response to ACTH and some impairment in response to "Metopiron", had a plasma sodium level of 135 m.Eq./L. before and after ACTH.

Girl no. 5, who had an impaired response to ACTH and almost no response to "Metopiron", showed a rise in plasma sodium from 140 to 154 m.Eq./L. during the ACTH test. A similar large rise from 138 to 150 m.Eq./L. was found in girl no. 20, who also had an impaired response to ACTH and no response to "Metopiron".

The lowest plasma sodium values were found in the boys, and they are best shown in tabular form:-

PLASMA SODIUM VALUES, BOYS

Patient	Plasma Sodium Before ACTH m.Eq/L	Plasma Sodium After ACTH m.Eq/L	ACTH test	"Metopiron"	Thyroid Function
14	132	135	Subnormal	No response	Clinically normal
16	132	-	Subnormal	No response	Normal
26	134	137	Subnormal	-	Normal
29	135	138	Normal	Good response	Hypo.
31	130	143	Normal	No response	Normal
35	135	135	Incomplete	Impaired	Normal
36	135	139	Normal	Almost no response	Normal

With the exception of patient no. 31, the lowest resting values for plasma sodium were found in those boys with grossly impaired adrenocortical function, nos. 14, 16 and 26, and in nos. 14 and 16, where the test was applied, there was no response to "Metopiron".

The control of sodium and water metabolism in the presence of hypopituitarism is not well understood. Some authors have attributed the hyponatraemia to water retention. MacLean and his colleagues (1957) investigated the sodium balance in nine patients who had hypophysectomy for breast cancer. In these patients,

hyponatraemia only occurred if the fluid intake was excessive in the presence of a low sodium intake.

Lipsett, West, MacLean and Pearson (1957) also compared the response to withdrawal of cortisone in hypophysectomised and in adrenalectomised patients. The former tolerated withdrawal better than the latter. It was considered that the existence of aldosterone secretion or the presence of diabetes insipidus did not account for this difference.

The increases in plasma sodium which occurred in girls nos. 5 and 20 after the administration of ACTH during the standard test cannot be explained on the action of ACTH alone. This rise was not seen in others with an impaired pituitary-adrenal axis (e.g. boys nos. 14 and 16), but it was present in boy no. 31, who showed a normal response to ACTH but no response to "Metopiron". Further studies are needed to elucidate this finding.

ANAEMIA IN HYPOPITUITARISM

Pallor and anaemia have frequently been recorded as part of the clinical description of hypopituitarism. Snapper, Groen, Hunter and Witts (1937) reported several instances of hypopituitarism associated with achlorhydria, hyperchromic anaemia and subacute combined degeneration. Escamilla and Lisser (1942) described anaemia and achlorhydria in hypopituitarism in which there was an average haemoglobin content of 65% and occasional eosinophilia. In 1948, Daughaday and his colleagues indicated the frequent finding in hypopituitarism of a striking pallor of the face sometimes described as "waxy-white" or "alabaster". This they attributed to loss of melanin from the skin and to the lack of the normal capillary flush on exposed skin. The pallor which Daughaday reported was more marked than would be expected from the degree of anaemia usually found in his cases, which was of moderate degree and which was characterised by normochromic cells. Macrocytosis was a rare occurrence. Cook et al. (1951), described unexplained refractory anaemia in two of seven hypopituitary patients. The anaemia was again of moderate degree, normochromic, and with mild leucopenia.

Hamilton Smith (1960) wrote of six cases of hypopituitarism which had presented as "anaemia refractory to the usual haematinics". Treatment with iron, vitamin B_{12} and folic acid for

periods of one to ten years was unavailing until the evidence of hypopituitarism was recognised. In Smith's series, the erythrocytes were normocytic and normochromic, except in one instance in which moderate anisocytosis and poikilocytosis and a few macrocytes were found. In a second case there was a moderate degree of hypochromia. Normoblastic erythropoiesis was seen on examination of the bone marrow. The peripheral white count ranged from 4,700 to 9,100 cells per cubic millimetre. One patient had a relative lymphocytosis. Two others in whom the test was performed showed free hydrochloric acid in the gastric contents.

Two girls in the present study (patients 9 and 15) were first seen because of their small size. Clinical and biochemical examination revealed the following abnormalities:- height percentile ratings of below three, retarded bone age, marked sensitivity to insulin, hypoglycaemia (no. 15) and anaemia. Both children showed a good response to ACTH during the standard test but patient no. 15 showed some impairment of response to "Metopiron". The "Metopiron" test on patient no. 9 could not be interpreted as one of the urine specimens was accidentally lost. There was no evidence of thyroid dysfunction nor was there any impairment of fat absorption on a five-day balance (Baldwin et al., 1962). In patient no. 9 attempts to treat the anaemia with iron were unsuccessful.

The bone marrow findings, particularly in patient no. 15,

were similar to those encountered in hypophysectomised rats, namely, a hypocellular marrow and pancytopenia. Although the anaemia of hypopituitarism is well-documented, the mechanism is obscure. A number of investigators (Garcia et al., 1951; Contopoulos, et al., 1953, 1954; and Van Dyke, et al., 1954, 1957), have postulated the existence of a simple pituitary erythropoietic factor with the bone-marrow as target-organ. However, Gordon (1959), in an extensive review of the literature, provided abundant evidence against this theory.

The anaemia of hypopituitarism has been fully corrected in rats by the combined use of cortisone, thyroid and testosterone (Huguley, 1960). Similarly in human subjects, cortisone, thyroid and growth hormone have been effective (Crafts and Meineke, 1959).

It is possible that the clinical findings in the children under discussion could be entirely due to growth hormone deficiency, but there is no satisfactory explanation for the impaired response to "Metopiron" in patient no. 15. The action of growth hormone on erythropoiesis is not known but it is partially effective in restoring the anaemia of hypophysectomised rats (Huguley, 1960).

SOME ORGANIC LESIONS ASSOCIATED WITH ENDOCRINE DYSFUNCTION

Two boys in this study were found to have suprasellar tumours. Patient no. 2 had a cyst of Rathke's Pouch, and patient no. 30 had radiological evidence of a craniopharyngioma. A third boy (no. 8) was first admitted in infancy because of anaemia which was diagnosed as erythrogenesis imperfecta. Both no. 2 and no. 8 were found to be dwarfed on clinical examination, and both were well below the third percentile for height. Patient no. 30 was not dwarfed but his endocrine status was evaluated before operation and he subsequently presented an interesting disturbance of growth.

Patient no. 2 and no. 8 showed clinical and biochemical evidence of panhypopituitarism, whereas no. 30 showed no clinical evidence of endocrine dysfunction apart from enlargement of the penis and testes. Biochemical investigation in this child revealed some degree of insulin sensitivity, using a very small dose of insulin (0.05 units/kg. body weight), and raised level of urinary 17-ketosteroids. In addition, no. 8 was slate-grey in colour and there was histological evidence of iron deposition in the skin. This child had received multiple transfusions of packed red blood cells from infancy and it seemed possible that his hypopituitary state was due to deposition of iron in or around the hypophysis.

In the two years before operation patient no. 2 showed a

Patient no. 2. Male. 12 years

STANDING HEIGHT BEFORE AND AFTER OPERATION

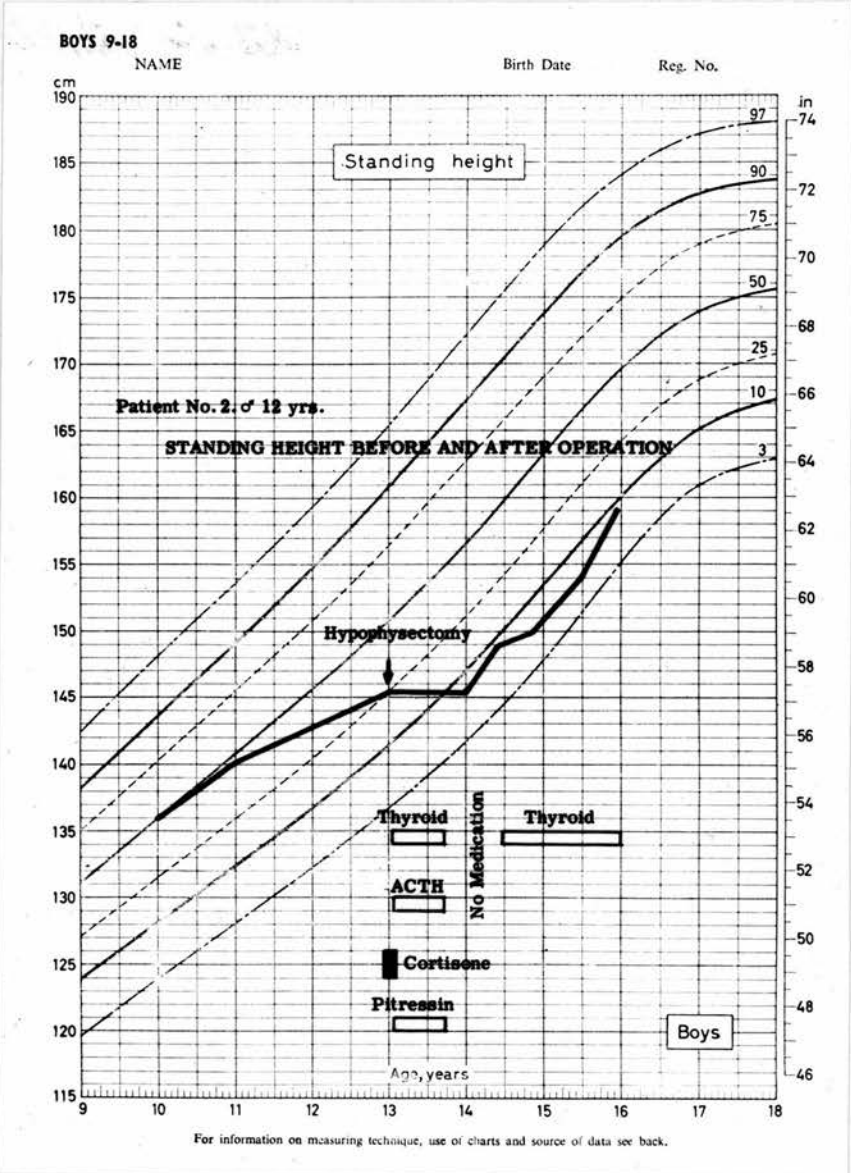

Fig. 8.

gradual slowing down of growth (measured by standing height, Fig. 8), and this remained at a constant value during treatment with cortisone, ACTH, thyroid and pitressin immediately before, during and after operation. When all therapy was stopped, the boy grew one inch in height in the succeeding six months and it seems probable that this retardation of growth was due to the influence of adrenal steroids produced by exogenous ACTH. An attempt was made to remove the iron deposits in patient no. 8 by means of Desferrioxamine (Ciba Laboratories Ltd.), a reported specific iron-chelating agent, but preliminary studies were not encouraging (Dr. R.Sephton Smith).

Although the height measurements of patient no. 30 are not available, this child grew very rapidly until at the age of nine years he had a skeletal age of twelve years six months. There were signs of puberty, except that tooth eruption was about normal for chronological age and there was no axillary hair. The urinary gonadotrophin activity was estimated at 3.2 units HMG 20A in twenty-four hours, but the significance of this was not clear in view of the absence of normal values in children when employing this method. According to Tanner (1962), accurate assessment of the value of dental maturity is not possible because it has not been sufficiently used as a measure of physiological maturity at adolescence. However, it seems that development of the teeth, and in particular, the rate of maturation, is more closely associated

with skeletal age and secondary sex characters at adolescence, than before. Demisch and Wartmann (1956) found a partial correlation between skeletal age (Greulich-Pyle) and the stage of third mandibular molar development when chronological age was held statistically constant. The absence of axillary hair in the presence of a pubic hair rating of stage 4 (Tanner, 1955), is unusual, but the time of appearance of axillary hair is variable.

The cause for this precocious puberty is not known but it may be related to a disturbance of hypothalamic function. Precocious puberty has been produced experimentally in some species by anterior hypothalamic lesions. Methods of recording have shown that there is activation of the anterior and lateral hypothalamus during spontaneous ovulation in rodents with regular menstrual cycles, or following vaginal stimulation in oestrous cats. The activated areas are near the median forebrain bundle (Greer, 1960). Complex endocrine changes ranging from panhypopituitarism to precocious puberty have also been described in lesions of the hypothalamus with an anatomically intact pituitary gland (Bauer, 1954).

In patient no. 2 there was evidence of restoration of pituitary function following operation, but on testing the adrenocortical response to ACTH it was found that the response was less than when the child had been receiving thyroid. It is well documented that surgical hypophysectomy in man is followed by a

fall in indices of urinary gonadotrophin excretion, thyroid function and 17-ketosteroid excretion (e.g. Luft and Olivecrona, 1953). At autopsy, atrophy of adrenals, gonads and thyroid closely parallels that seen in hypophysectomised rats (Shimkin et al., 1952). As total hypophysectomy in man is technically difficult, residual hypophyseal cells not infrequently remain. In partially hypophysectomised patients there is a restoration of thyroid function after an initial fall (Elden and Kummer, 1943), menstruation may return and pregnancy occur (Luft and Olivecrona, 1953). Similarly, in Sheehan's syndrome (Sheehan, 1939) where residual hypophyseal cells remain, spontaneous recovery may occur. This is accelerated if the patient subsequently becomes pregnant (Sheehan and Murdoch, 1939).

CASE SUMMARIES

Patient No. 2:

This boy, aged twelve years ten months, was admitted with a six-month history of temporal headaches which recurred every six weeks and which lasted for two to ten days. The headaches were very severe at times, he felt ill, became "greenish" in colour, and he was very drowsy. The patient was in good general health between headaches, and there was no vomiting unless he was forced to eat. He attended an optician who found no visual disturbance

to account for the headaches. The boy was slightly anaemic for six months prior to admission.

X-ray examination of the paranasal sinuses at a local hospital revealed an abnormally large pituitary fossa and an extremely thin dorsum sella. This was confirmed by further skull X-rays. Clinical examination of the central nervous system was entirly normal, but accurate assessment of the visual fields showed a definite loss of the upper left temporal region. He was transferred to The Hospital for Sick Children for further investigation and treatment.

On general examination the boy was found to be pale and small for his age, with loss of hair on the skin. He had infantile genitalia and the left testis was not palpable in the scrotum. Examination of the central nervous system showed no abnormality and the visual fields were full to confrontation. Perimetry confirmed the loss in the left temporal region which had been reported previously.

Lumbar air-encephalography and "Myodil" ventriculography (Dr. R.D.Hoare) revealed the presence of a suprasellar space-occupying lesion which projected into the basal cisterns and caused a filling defect of the anterior part of the third ventricle. There was no intracranial calcification. Films taken at another hospital when the child was six years old showed a normal sella.

Chemical and bacteriological examination of the cerebro-spinal fluid, and the total and differential white blood count, were normal. Haemoglobin was 11.4 g./100 ml.

Biochemical investigation of the endocrine system before operation showed a response to exogenous ACTH which was typical of that seen in patients with hypopituitarism. The resting levels of urinary 17-ketosteroids and total 17-hydroxycorticoids were about one-tenth of normal. The ability to raise the 17-ketosteroids after stimulation was also poor.

In addition, there was increased insulin sensitivity and a high, flat glucose tolerance curve. Blood cholesterol was at the upper level of normal, determinations of basal metabolic rate were significantly low, but the serum protein-bound iodine value of more than 10 μg./100 ml. was invalid because of previous "Myodil" ventriculography.

The child was therefore treated with cortisone before, during and after operation. A purplish tumour mass was found to occupy most of the pituitary fossa and the sella turcica appeared completely empty after removal. The lesion was diagnosed histologically as a cyst of Rathke's Pouch by Dr. M.Bodian and by Professor T.Crawford.

The child's post-operative condition was excellent but he drank large quantities of fluids and he did not concentrate urine when the intake was restricted from 280 fluid ounces to 50 fluid ounces each day. No pitressin was given. ^{131}I studies at University College Hospital showed a low thyroid uptake. The child was transferred to The Royal Marsden Hospital for radiotherapy on a maintenance dose of 12.5 mg. cortisone acetate twice daily, and Thyroid B.P., grains $\frac{1}{2}$ each day.

One month after operation and radiotherapy the patient was readmitted for endocrine assessment. He had no complaints apart from polyuria, and on examination he was pale, of small size, with dry skin and fullness of the face. There were no signs of pubescence and the left testis was not palpable in the scrotum. Trial doses of ACTH were given and the urinary output of 17-ketosteroids and total 17-hydroxycorticoids was determined. On stopping the ACTH it was found that the adrenal stimulating power of the pituitary was inefficient but there was evidence of restoration of pituitary function. The adrenocortical response to the standard test with exogenous ACTH was normal. The dose of ACTH was adjusted to 15 units daily on the basis of urinary steroid output and the child was discharged on a fifteen-hundred Calorie diet to prevent excessive weight gain, 15 units of ACTH gel daily by intramuscular injection, Thyroid B.P. 1 grain each day and one capsule of Pitressin snuff each day in divided doses.

Seven months later all therapy was stopped and he was admitted for endocrine assessment six months after that. On measurement at the growth clinic (Dr. J.M.Tanner) it was found that the child had grown one inch in height during the six months without therapy and an ACTH test showed a good response to stimulation but considerably less than that obtained when the child was on treatment three months after operation. The striking clinical and biochemical features at this time were those of hypothyroidism.

Patient No. 30:

This boy, aged six years and ten months, was well until a few months before admission when he complained of headaches and fits which were diagnosed as Petit Mal. He was treated with "Tridione". The fits continued and became more severe. They were of short duration (five to ten seconds) and sometimes the child had five or six fits a day, with intervals of half to three quarters of an hour between each episode. Usually they occurred first thing in the morning.

During a fit, the boy would hold his head still, stare, then drop his head. This was followed by normal behaviour. He was incontinent of urine during a fit on three occasions only. There was frequent vomiting in the two weeks before admission, which was forceful and unrelated to food. The child felt sick for only a few seconds before vomiting.

General examination was normal apart from moderate enlargement of the penis and testes, with some proliferation of pubic hair. On examination of the central nervous system the patient was found to have a head circumference of 57.5 cm. (23 inches), bilateral constriction of the visual fields, and marginal blurring of the discs associated with moderate hypermetropia (Mr. J.H.Doggart). X-ray films of the skull showed an area of calcification 1 cm. in diameter in the region of the right posterior clinoid process, and a lumbar air-encephalogram (Dr. R.D.Hoare) confirmed the presence of a large suprasellar tumour with hydrocephalus.

On biochemical investigation there was some degree of insulin sensitivity after a small dose of insulin (0.05 units per kg. body weight), and resting values of urinary 17-ketosteroids were raised. There was a good response to exogenous ACTH and the serum protein-bound iodine level was normal.

Treatment consisted of aspiration of the tumour (Mr. K.Till) followed by radiotherapy.

The child was not brought to the Hospital until ten months after operation. He was very well with no recurrence of fits. Physical examination revealed a moderate degree of blindness in the right eye and pallor of both optic discs. The penis and testes had become more enlarged since operation. There was no axillary

hair but pubic hair was present and urinary gonadotrophins were estimated at 3.5 units HMG 20A in twenty-four hours, although this value could not be fully interpreted in the absence of normal ranges in childhood by this method.

Ten months later the child was seen by Dr. J.M.Tanner when it was found that his voice had broken and he had grown at "an astonishing rate" since operation. The child's genital development and growth of pubic hair were rated as stage 4 (Tanner, 1955). The testes were adult in size and consistency. He had erections but he was not emotionally disturbed in the presence of girls. Examination of the central nervous system showed no evidence of recurrence of the tumour.

The child continued to grow very rapidly and when seen six months later he appeared to be in a normal adolescent "growth-spurt" but tooth eruption was about normal for chronological age. The voice was deep and there was a light growth of hair on the upper lip. There was no axillary hair but the pubic hair was between stages 4 and 5. The penis was adult in size.

The following table lists estimations of skeletal age

(Greulich and Pyle, 1959) against chronological age:-

Patient No. 30.

Chronological age (years)	Skeletal age (y = years) (m = months)
6.89	7y 0m*
7.8	9y 3m
8.5	11y 0m
9.0	12y 6m

* Estimated in the Department of Radiology.

CHRONIC REGIONAL ILEITIS AND HYPOPITUITARISM

The relationship between malnutrition and hypopituitarism has been well-described in experimental animals (Bennett, Li and Evans, 1948; Li, Simpson and Evans, 1949; Samuels, 1950; D'Angelo, 1951) and in human subjects, chiefly the result of war privations (McCullagh and Tupper, 1940; Sydenham, 1946; Thorn et al, 1950). In 1959, Fletcher and Brown reported impaired pituitary function in ten women who had severe loss of weight which could only be ascribed to anorexia for which no organic cause could be found.

Impaired pituitary function may also result from primary disease of the alimentary tract. Mickerson (1960) described defective end-organ response in a series of patients with steatorrhoea. The author was unable to attribute his findings to decreased caloric intake because he maintained that intestinal absorption in steatorrhoea is considerably greater than that in people who exist under famine conditions.

Several instances of dwarfism have been reported in children with chronic regional ileitis (Logan and Brown, 1938; Tanner, 1939; Crohn and Yunich, 1941; Alvarez, 1945) and in each case, intestinal symptoms comprised the chief complaint. Sobel, Silverman and Lee (1962) reported two children with chronic regional ileitis who presented with slow growth and who were at first considered to be hypopituitary dwarfs. These writers stressed the difficulty in diagnosis which is exemplified by the following case.

An eight year old boy (patient No.20) was first seen because of alimentary symptoms, but radiological examination after barium meal showed no abnormality. In view of his small size, retarded skeletal age, anaemia and an impaired fat absorption of 91.8%, it was decided to treat him initially on a gluten-free diet.

The child was seen nine months later when there was little change in his physical condition. A repeat fat balance showed 94% absorption and there was clearcut evidence of impaired pituitary function on endocrine investigation. The nature of the intestinal complaints remained obscure, and another barium meal examination was carried out. Before his next outpatient visit the child was admitted with a diagnosis of appendix abscess which was not upheld by the examining surgeon. He made the clinical diagnosis of regional ileitis which was supported by radiological examination, and by the findings at laparotomy.

The mechanism of the relationship between regional ileitis and pituitary function is not known. Crohn and Yunich (1941) mentioned that where the disease begins before or at puberty, there is considerable retardation of secondary sexual development. These authors described one boy who responded to medical treatment and who gained weight and who showed normal secondary sexual maturation. Sobel, Silverman, and Lee (1962) considered the hypopituitary state to be the result of ileitis, both patients described by them improved dramatically after surgical treatment. In one child, growth was again retarded when the disease recurred.

Pituitary dysfunction in chronic regional ileitis may be the result of diminished caloric intake or deficiency of a specific dietary factor. Maddock and Heller (1947) provided evidence that there might be a failure of the secretory mechanism of pituitary hormones early in malnutrition. Samuels (1948) showed that in rats, where gonadal atrophy occurred there was a negative nitrogen balance. In further studies, he attempted to answer the question whether lack of a specific amino acid was responsible, or whether there was a general inability to utilise amino acids. He studied deficiency of tryptophan, and found in the rat, that gonadal atrophy occurred in the absence of this amino acid even though total amino acid and caloric intake were unchanged. In these animals both the thyroid and pituitary were small at autopsy.

In this child there was impaired fat absorption before the administration of a gluten-free diet, one must therefore consider the possibility of deficiency of fat soluble vitamins in view of the effect of certain vitamins on the ability of the end organs to respond to gonadotrophic hormones (Mason, 1944; Hertz and Tullner, 1949).

The maintenance of a negative nitrogen balance even though small will result in impaired pituitary function. It is tempting to attribute this child's endocrine dysfunction to malnutrition but failure of growth was first noted on first appearance of symptoms. One may question the reliability of the parents as witnesses but in the absence of exact physical measurements this argument must remain unsettled.

It seems possible that all patients with chronic regional ileitis have impaired pituitary function and that the partial therapeutic success of ACTH or corticosteroids is primarily due to restoration of a deficiency.

SECTION 2.

METABOLIC STUDIES WITH

HUMAN GROWTH HORMONE

METABOLIC STUDIES WITH HUMAN GROWTH HORMONE

It is a cardinal principle of medical treatment that therapeutic trial of a specific agent is obligatory especially in those cases where long-term administration is envisaged. This is particularly applicable in hormone replacement therapy where the substance to be given may have potent metabolic effects.

The studies to be described were undertaken, prior to treatment, in four children in an attempt to evaluate the efficacy of a Human Growth Hormone preparation. These children were considered to have a probable deficiency of that hormone and their mode of selection for metabolic investigation is discussed elsewhere in this thesis.

Reifenstein, Albright and Wells (1945) presented a survey of the methods then in use in The Metabolic Unit, The Massachusetts General Hospital, in which they expressed the hope that some of the methods might be adopted by others in order to establish uniformity in the field. Most metabolic work is now based upon the pioneer studies of this group.

Methods of Conducting Balances:

The present investigations formed part of the work of the Routine Laboratory, Department of Chemical Pathology, The Hospital for Sick Children. In the absence of a special metabolic ward it is the practice for metabolic balances to be carried out wherever

a child is situated. We have found this system admirable. The balance is under the immediate supervision of the Ward-Sister or Staff-Nurse, in close co-operation with the Diet Kitchen and the Laboratory.

It is our practice to explain the nature and purpose of the study to the parents and to the child where this is possible. It is as important to gain the co-operation of the child in metabolic work as it is in the physical examination. A written schedule with a brief explanation of the studies to be undertaken is distributed to the departments concerned before the balance is begun.

Diet Shortly after admission, the child's dietary requirements were assessed by the Dietitian in conjunction with the nursing staff. During an interval of 5-7 days before the balance regime, the child was allowed freedom of choice from those foods which could be deep-frozen. Except in the case of patient number 24, (C.L.), food was obtained in bulk at the start of the investigation, distributed into meals and deep-frozen.

Food Balances were begun at 10 a.m. The child was given no food except that supplied by the Diet Kitchen, although distilled water was given in free amount. No tooth-paste was used throughout the study period; teeth were cleaned with distilled

water only. A duplicate of all food and fluids given to the child was sent to the laboratory daily, except where the Diet Kitchen carried sufficient deep-frozen stocks, thereby limiting the analysis to one day's meals. Milk samples and perishable foodstuffs which could not be deep-frozen, and where composition was subject to daily variation, were sent to the laboratory each day.

At the end of each balance period the food and fluids were pooled and homogenised in a plastic bucket with a Silverson Laboratory Mixer-Emulsifier ("Silverson"); the homogenate was made up to a suitable volume (usually 5-10 litres) with distilled water. This was done by allowing the "Silverson" to run until a homogenous fluid with no lumps was obtained. It was then stopped, removed from the bucket, and the volume was made up to the nearest mark. The "Silverson" was then replaced and run until the fluid was thoroughly mixed. Milk was homogenised for only a few seconds to prevent the formation of a thick froth.

Sampling of Homogenate While the "Silverson" was running, 200 ml. of homogenate were transferred to a beaker and a further 100 ml. were placed in a polystyrene pot for storage at -20°C. The pot was not filled more than two-thirds its volume because of expansion on freezing.

Black-Ashing The 200 ml. of homogenate in the beaker were

stirred continuously during the slow addition of 100 ml. concentrated sulphuric acid (M. and B., R.R. Low in nitrogen). Where frothing occurred, the speed of stirring was increased. Stirring was continued until room temperature was reached. Material which had been properly ashed was jet black, where it was brown more sulphuric acid was added. The black liquid was made up to 500 ml. and mixed. This was known as the "black-ashed material" and an aliquot of approximately 100 ml. was stored in a polystyrene pot at room temperature.

Rejects Rejects were sent to the laboratory from the Diet Kitchen at the end of each period. They were treated exactly as diets.

Vomit Wherever possible, vomit was sent for analysis and the specimens were included in the rejects.

Urine Urine was collected over 24-hour periods from 10 a.m. At this time on the first day of the balance, the patient emptied his bladder and this specimen was discarded. All urine passed up to and including the specimen at 10 a.m. the next day was saved in polythene bottles containing 5 ml. of glacial acetic acid. These were labelled with the child's name, the dates of both days covered by the collection, and the exact time of commencement. If any specimen was accidentally lost, details of the time passed and an estimate of the volume (where possible) were sent to the laboratory.

Stool The collection of urine and faeces in children, with particular reference to infants and incontinent patients, has been described by Baldwin et al. (1962). The method of stool collection in this study was based on that reference. Each stool was collected separately in polythene sheeting and the complete specimen was placed in a waxed paper carton which was labelled with the child's name, the date and the time at which the stool was passed. Record sheets were issued to the ward for this purpose. For timed faecal collections it was necessary to give a marker. Charcoal was unsuitable, therefore for those children over one year of age, carmine powder grains 5 and Edicol Supra Blue E.G. (Imperial Chemical Industries Ltd.) 1 mg./kg. body weight were given alternately. Faecal collections were begun at the first appearance of the dye. Stools were saved until the second marker appeared, and the stools containing the second marker were sent to the laboratory. The marked stool was not included in the analysis unless the balance was continuing, and then it became the first specimen of the next period.

Before homogenising, all specimens of faeces were checked from laboratory records against those of the ward. All the stools for each balance period were bulked and homogenised; the known volume was such that a homogenate of manageable consistency was obtained. 200 ml. of this was black-ashed and stored using the method which was applied to the diets. A new model of the

Silverson homogeniser is now available which is particularly suited for faeces. It has a totally enclosed rotor arm thereby minimising aerosol formation.

METHODS OF ANALYSIS

Procedure for Digesting Calcium and Phosphorus

Reagents:

1. Concentrated sulphuric acid S.G. 1.84 (Analytical Reagent (A.R.)).
2. Hydrogen peroxide 100 vols. (A.R.). Stored at 4°C.
3. Sodium acetate (A.R.) 20% w/v anhydrous salt in aqueous solution.
4. Bromocresol green (B.D.H.).
5. Ammonia. 0.880 (A.R.).
6. Glacial acetic acid (A.R.). 33% v/v in aqueous solution.

Method: (1-5 ml.)* of black-ashed material or urine was pipetted in duplicate into micro-Kjeldahl flasks calibrated at 20 ml. 1 ml. concentrated sulphuric acid and a 2 mm. diameter glass bead were added. The flask was heated on a digestion rack until the sulphuric acid was anhydrous and extreme care was taken to prevent fluid loss through bumping. Where large amounts of fat were present the food blackened and appeared to absorb sulphuric

* Stool + urine: 1 ml. Stool: 2 ml. Urine: 5 ml.
Black ashed diet, rejects, milk: 5 ml.

acid. When this happened, a further 1 ml. of acid was added.

When the acid was anhydrous and refluxing gently, the flask was removed from the flame and a few drops of hydrogen peroxide were added directly to the hot acid, with the mouth of the tube pointing away from the operator. The flask was replaced on the digestion rack and boiled until the water was driven off. The addition of peroxide was repeated until the fluid remained clear after fifteen minutes' boiling. The tubes plus contents were allowed to cool, 5 ml. of distilled water were added, the contents were mixed by swirling and allowed to cool again. 1 ml. of 20% sodium acetate solution and 4 drops of bromocresol green were added. 0.880 ammonia was then added until the indicator turned blue-green (pH 4.5 - 5.0) when compared with a standard. Where necessary the pH was adjusted with 33% acetic acid and the contents were made up to the 20 ml. calibration and mixed.

This was known as the "blue-green liquid" and was used for calcium and phosphorus estimations.

Procedure for Estimating Calcium

Reagents:

1. Standard Calcium Solution 10 mg./100 ml. 250 mg. of calcium carbonate (A.R.) were dissolved in 6 ml. N/1 hydrochloric acid added dropwise. The mixture was gently heated on a boiling-

water bath until all the hydrochloric acid had vaporised. (The vapour was tested with blue litmus paper). The solution was cooled anddiluted to one litre.

2. Ammonium oxalate saturated solution. This contained approximately 45 g./L. ammonium oxalate (A.R.) and was filtered before use.
3. Dilute ammonia. 20 ml. 0.880 ammonia (A.R.) diluted to one litre.
4. Sulphuric acid (A.R.), approximately N/1 2.8 ml. concentrated acid/100 ml.
5. Stock potassium permanganate solution N/10 B.D.H. Standard.
6. Working potassium permanganate N/50. This was made by diluting the stock solution 1:5. It could be used for one week if stored in a dark covered bottle. The normality was not adjusted.

Method: 10 ml. of the "blue-green liquid" were transferred to a 50 ml. conical centrifuge tube. 1 ml. of saturated ammonium oxalate was added to each tube and to 5 ml. of the standard calcium solution in a similar tube. A "blank" was also set up which consisted of 5 ml. of water and 1 ml. of ammonium oxalate. The tubes were shaken to mix and left overnight at room temperature to precipitate the calcium oxalate.

The tubes were spun at 2,500 r.p.m. for 30 minutes. The supernatant was removed by gentle suction with a hooked Pasteur

pipette, and the precipitate was washed three times with 5 ml. of dilute ammonia. The latter was added gently from a wash bottle with a fine jet. The sides of the tube were washed down without disturbing the precipitate. The tubes were centrifuged for 10 minutes at 2,500 r.p.m. after each wash and the supernatants were removed as before.

Finally, the calcium oxalate was dissolved in 2 ml. of N/1 sulphuric acid at 60-70°C and was titrated with N/50 potassium permanganate while still hot. A 5 ml. burette graduated in 0.01 ml. was used. Duplicate titrations were accepted within 0.04 ml.

Calculation:

$$\frac{\text{Titration of test}}{\text{Titration of standard}} \times 0.5 \times \frac{20}{\text{Volume of "blue-green liquid" used}} \times \frac{\text{Total volume black ashed material}}{\text{Volume of black ashed material used}} \times \frac{\text{Volume of homogenate made}}{\text{Volume of homogenate used}} = \text{mg. Calcium in total original sample.}$$

Calcium Determination by Flame Photometer

Instrument: "Eppendorf" flame photometer.

Fuel/gas systems: Calcium, air, 0.5 mg./cm.^{2}/acetylene, 0.42 L./minute.

Sodium, air/propane air 0.5 mg./cm.^{2}/propane mm/WS.

Filters: Calcium band 620 mμ.

Sodium line 589 mμ.

Solutions:

1. Calcium stock standard (20 m.Eq. calcium/L.)

   1.000 g. dry calcium carbonate (A.R.) was dissolved in 25 ml. N/1 hydrochloric acid and made up to one litre with de-ionised water.

2. Working calcium standard (0.2 m.Eq. calcium/L.). 1 ml. of stock solution was diluted to 100 ml. with de-ionised water.

   Ten scale divisions on the photometer were equivalent to 0.2 m.Eq. calcium/L.

3. 0.1 N hydrochloric acid.

4. Sodium stock standard (100 m.Eq. sodium/L.).

   5.85 g. dry sodium chloride (A.R.) was dissolved in de-ionised water and made up to one litre. Dilutions of this standard were made to cover a range of up to 50 m.Eq. sodium/L. A calibration curve was constructed from which the sodium content of the samples could be determined.

Experimental: The instrument was shown to behave linearly to calcium concentrations up to 0.5 m.Eq. calcium/L. At the concentrations of calcium present in the diluted samples (below 0.2 m.Eq./L.) it was found that sodium was the only interfering substance. For each milliequivalent of sodium present in the diluted sample it was necessary to deduct 0.002 m.Eq. calcium from the apparent calcium content.

Preparation of samples: 10 ml. aliquots of homogenised food or faeces were transferred to 100 ml. silica beakers. Urine was thoroughly mixed and 20 ml. samples were pipetted into silica beakers.

Ashing: Samples were slowly dried on a sand bath before ashing at 420°C in a muffle-furnace overnight.

The residue was heated with about 10 ml. of N/10 hydrochloric acid for ten minutes on a sand bath. When cold, the extract was filtered through an acid-washed Whatman No. 42 filter-paper into a 25 ml. volumetric flask for food extracts or a 50 ml. volumetric flask for faecal extracts.

The paper and funnel were thoroughly washed with portions of N/10 hydrochloric acid and the extract was made up to the required volume.

Estimation: Samples were further diluted so that the estimated concentration of calcium in the sample was below 0.2 m.Eq./L. The calcium emission from the samples was compared with that from a standard solution containing 0.2 m.Eq. calcium/L.

The sodium content of the diluted samples was estimated and the apparent calcium content was corrected for sodium enhancement.

Procedure for Estimating Phosphorus

Reagents:

1. Standard Phosphorus Solution - 25 mg./100 ml. 1.0975 g. potassium dehydrogen phosphate (A.R.) was dissolved in ion-free water and diluted to one litre. A few drops of chloroform as preservative were added. The solution was stored at $4^{o}C$.
2. Briggs Molybdate Solution. 25 g. of crushed ammonium molybdate (A.R.) were dissolved in about 300 ml. of water. 75 ml. of concentrated sulphuric acid (A.R.) were added to 125 ml. of water and when cool this was mixed with the ammonium molybdate solution and diluted to 500 ml.
3. Hydroquinone 1% w/v solution. This was made freshly for each set of determinations.
4. Sodium sulphite (A.R.) 42% w/v. 42 g. Na_2So_3, 7 H_2O were dissolved in warm water and diluted to 100 ml. when cool. Care was taken not to overheat.

Method: Sufficient 50 ml. volumetric flasks to allow two for each test, one for a standard and one for a blank, were taken. The following solutions were added to each flask and the contents were mixed after each addition:-

40 ml. water.

2.5 ml. Briggs Molybdate Solution.

0.5 ml. Hydroquinone.

0.5 ml. Sodium sulphite.

1 ml. of standard phosphorus solution was added to one flask and an aliquot* of blue-green liquid" to the others so that a colour approximately that of the standard was obtained. The solutions were made up to the calibration, mixed by inversion and allowed to stand for 60 minutes at room temperature; they were then read on an EEL colorimeter in 1 cm. cells using a 607 filter.

Calculation:

$$\frac{\text{Reading of test}}{\text{Reading of standard}} \times 0.25 \times \frac{20}{\text{Volume of "blue-green liquid used}} \times \frac{\text{Total volume black-ashed material}}{\text{Volume of black-ashed material used}} \times \frac{\text{Volume of homogenate made}}{\text{Volume of homogenate ashed}} = \text{mg. Phosphorus in total original sample.}$$

* Stool, urine: 2 ml.

Milk, diet, rejects: 4 ml.

Alternative Procedure for Estimating Phosphorus

The method of Gomorri (1942) was followed but N/10 HCl was used instead of 10% w/v trichloracetic acid.

Reagents:

1. N/10 Hydrochloric acid (A.R.).
2. Ammonium molybdate solution. 7.5 g. ammonium molybdate (A.R.) was dissolved in about 200 ml. of water. 100 ml. 10 N sulphuric acid was added and the solution made up to 400 ml with water.
3. "Metol" (p-dimethylaminophenol sulphate). 1 g. was dissolved in 100 ml. 3% w/v solution of sodium bisulphite (A.R.).
4. Standard phosphate solution (5 mg. phosphorus/100 ml.). 0.2197 g. potassium dihydrogen phosphate (A.R.) (KH_2PO_4) was dissolved in water and made up to one litre. A few drops of chloroform were added as preservative.

Method: Aliquots of the hydrochloric acid extracts of food and faeces were made up to a volume of 5 ml. with N/10 hydrochloric acid. Urine was diluted 1:25 and 1 ml. of the diluted urine was made up to 5 ml. with N/10 hydrochloric acid.

Ammonium molybdate (1 ml.) was added to each tube and after thorough mixing, "Metol" (1 ml.) was added at half-minute intervals. A blank and standard (which contained 0.5 ml. standard phosphate

solution) were included in each set of determinations. The solutions were allowed to stand at room temperature for thirty minutes. The colour was read at 607 mμ in an EEL colorimeter, using 1 cm. cells.

Calculation:

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.025 \times \frac{\text{Volume of acid extract made}}{\text{Volume of acid extract used}} \times \frac{\text{Volume of homogenate made}}{\text{Volume of homogenate ashed}} = \text{mg. Phosphorus/specimen.}$$

Procedure for Estimating Nitrogen

Reagents:

1. Boric acid + indicators.

   Solution A: 100 mg. phenolphthalein to 100 ml. with 96% v/v ethanol.

   Solution B: 33 mg. Bromocresol green, 66 mg. Methyl red } to 100 ml. with 96% v/v ethanol.

   5g. Boric acid
   200 ml. Industrial methylated spirit
   700 ml. distilled water
   35 ml. solution A
   10 ml. solution B } These were mixed to dissolve and diluted to one litre with water.

2. Sulphuric acid N/70. This was prepared from B.D.H. Standard

N/7 acid or by dilution of concentrated sulphuric acid with standardisation against boiling sodium carbonate.

3. Sodium hydroxide (A.R.) 40%. This was stored in an air-tight polythene bottle. If a high blank was obtained, ammonia-free air was drawn through the solution for several hours.
4. Selenium catalyst. Using a large "Amal" burner, 100 g. selenium metal (A.R.) was boiled with one litre concentrated sulphuric acid S.G. 1.84 (M. & B., low in nitrogen) until colourless. The solution tended to turn yellow on standing and where a precipitate formed the reagent was discarded.

Method: 1 ml. or a suitable sample* of black-ashed material or 0.20 ml. of urine was delivered in duplicate from an Ostwald pipette into a micro-Kjeldahl flask which contained a glass bead to prevent bumping. 1 ml. of selenium catalyst was added and the flask was heated on a micro-digestion rack so that the contents boiled vigorously without "bumping". Heating was continued until all the water was boiled off and the solution cleared. The solution was boiled for a further thirty minutes, cooled and steam-distilled in a Markham apparatus.

Calculation: 1 ml. N/70 acid ≡ 0.2 mg. nitrogen.

* The volume taken was such that a titration of between 2 and 10 ml. was obtained.

Procedure for Estimating Sodium and Potassium

These were estimated, in duplicate, on the unashed homogenate and on the urine directly.

Method: 25 ml. of homogenate was gently heated with 3 ml. concentrated nitric acid in a 50 ml. conical flask. This was done on a sand bath. The digest was brought to boiling point when a definite coagulase appeared (yellow solid in a yellow liquid). The flasks were cooled and the liquid was filtered through a fluted Whatman No. 42 filter paper into a 100 ml. volumetric flask. The conical flask and the precipitate in the filter-paper were washed three times with distilled water. The yellow solution was made up to 100 ml. with distilled water and then further diluted 1:25 to give a reading measurable on the flame photometer.

Urine was diluted 1:100 without digestion. The flame photometer was calibrated from 0-150 m.Eq./L. and these dilutions were usually suitable, although sometimes it was necessary to use others.

All glass-ware was well rinsed with ion-free water before use and checked on the flame photometer. A deflection of not more than two divisions was obtained with the sodium filter and the sensitivity at full-scale.

Calibration of the Flame Photometer

Reagents:

1. Stock Solution. 150 m.Eq./L. Sodium and Potassium. 8.775 g. sodium chloride (A.R.) and 11.2 g. potassium chloride (A.R.) were dissolved in water and diluted to one litre. The salts had previously been dried at 110°C overnight and allowed to cool in a dessicator.
2. Working Solution. The Stock solution was diluted 1:10 and 1, 2, 3, 4, 6, 8 and 10 ml. of this were diluted to 100 ml. (≡ 15, 30, 45, 60, 90, 120 and 150 m.Eq./L., respectively).

The photometer was set on 100 with the strongest working solution and all the others were read at this setting. The zero and full-scale deflections were checked between each reading. A graph of concentration against reading was plotted and the concentration of the test was read from this. This was multiplied by 100 for comparison with the stock standard.

The graph was drawn on two separate occasions to confirm the stability of the flame photometer.

CLINICAL TRIAL

Balance studies were carried out on four boys (patients 12, 24, 31 and 33). In the first three children no abnormality was found on physical examination or laboratory investigation, apart from small size, retarded bone age, and marked sensitivity to insulin. In addition to these anomalies, patient number 33 was shown to have impaired adrenal function. When these metabolic studies were carried out it was considered that patients 12, 24 and 31 exhibited possible growth hormone deficiency. However, in the light of further investigations on normal and dwarfed children, these patients also show some degree of impaired function of the pituitary-adrenal axis.

On completion of two five-day control periods, 10 mg. Human Growth Hormone (M.R.C. Raben preparation) were given by deep intramuscular injection at 10 a.m. on the first day of the third period. The balance was continued for two five-day periods after injection in patients 12 and 24. Unfortunately, patient 33 was not given a marker on the fifth day after growth hormone, and this period was extended to seven days. In patient 31, the urines on the fourth and fifth days after injection were pooled on the ward, therefore half the 48-hour value was taken for each day. The results of the studies in patients 12, 24 and 33 suggested that an additional five-day period after growth hormone might yield more information, and this was done in patient 31. Nitrogen, phosphorus (as phosphate), calcium, sodium, and

potassium were determined on dietary intake, faeces and urine on each child. Plasma electrolytes, blood urea, alkaline phosphatase, calcium, phosphorus and creatinine were also determined where practicable. A complete set of plasma values was not obtained in every instance because of the difficulty in collecting sufficient blood for determination by micro-method, particularly in patient 33 who was very small and who did not bleed readily from a finger prick.

The results are presented in diagrammatic form according to the method of Bassett (Bassett, personal communication quoted by Reifenstein, Albright and Wells, 1945). The scale for intake and balance in g./24 hours, mg./24 hours, or m.Eq./24 hours is given as the ordinate. The time-scale, in days, is given as the abscissa. The broken horizontal line at zero of the ordinate is the base-line to which intake and balance refer.

The intake is plotted as an area from the base-line towards the bottom of the diagram. The excretion is plotted from the bottom of the intake to the top of the diagram. The areas in black represent the faecal excretion during the five-day periods. The daily urinary excretions are plotted from the top of the faecal excretion to the top of the diagram. If the excretion does not reach the base-line, a white area is left between the excretion line and the base-line; this represents a positive

balance. If the excretion reaches the base-line, the balance is on equilibrium. If it exceeds the base-line, then this represents a negative balance. Reifenstein, Albright and Wells (1945) tried many different kinds of chart but Bassett's method allows one to focus on the balance, which is the most important aspect of the study. This is not so with other methods of presentation, where intake and excretion are plotted from the same base-line.

Interpretation:

Since dietary intakes are accurate within 10% in most instances (Reifenstein, Albright and Wells, 1945), any significant fluctuation in the overall balance must be more than 10%.

In the following charts (Figs. 9 to 32, inclusive) and tables (Tables 27 to 46, inclusive) the results of the balances and analyses on blood and plasma are presented.

PATIENT NO. 12 (M.F., MALE)

PLASMA VALUES AND BLOOD UREA

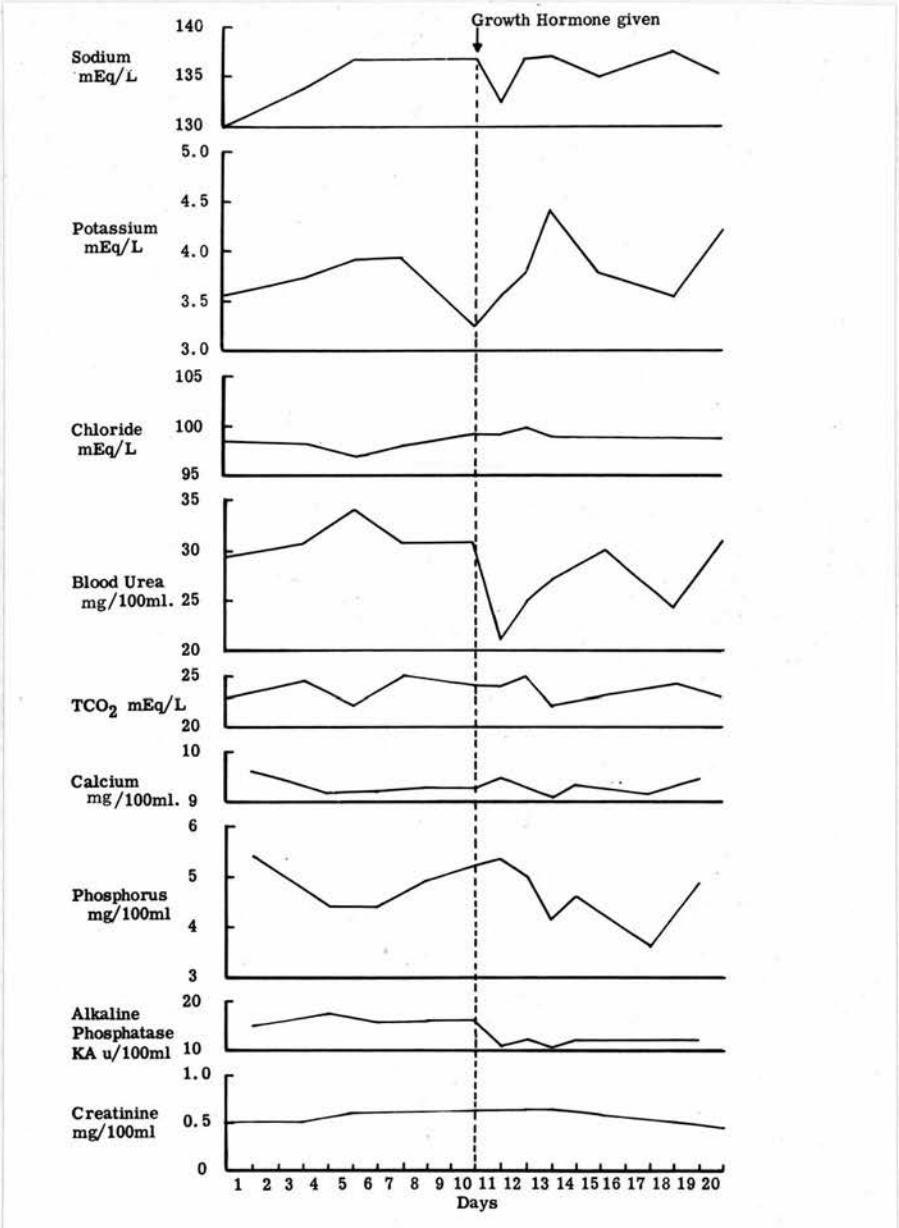

Fig. 9.

PATIENT NO. 24 (C.L., MALE)

PLASMA VALUES AND BLOOD UREA

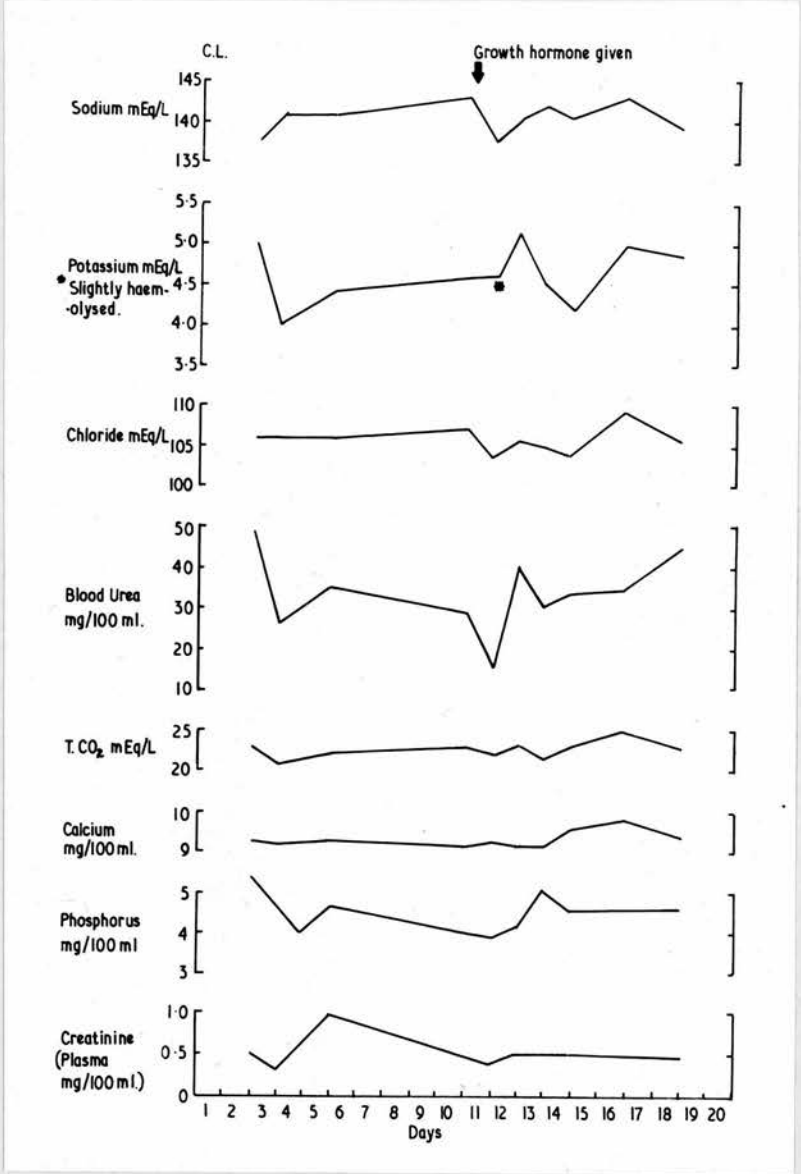

Fig. 10.

PATIENT NO. 31 (D.R., MALE)

PLASMA VALUES AND BLOOD UREA

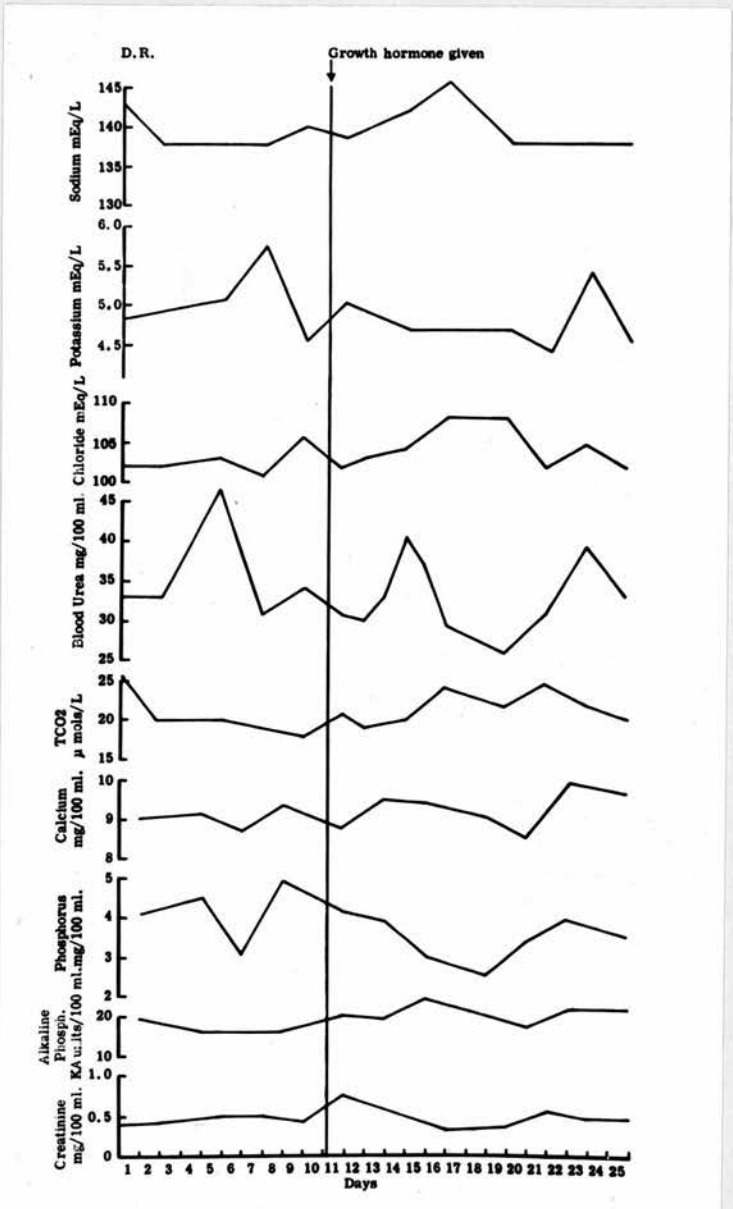

Fig. 11.

PATIENT NO. 33 (C.R., MALE)

PLASMA VALUES AND BLOOD UREA

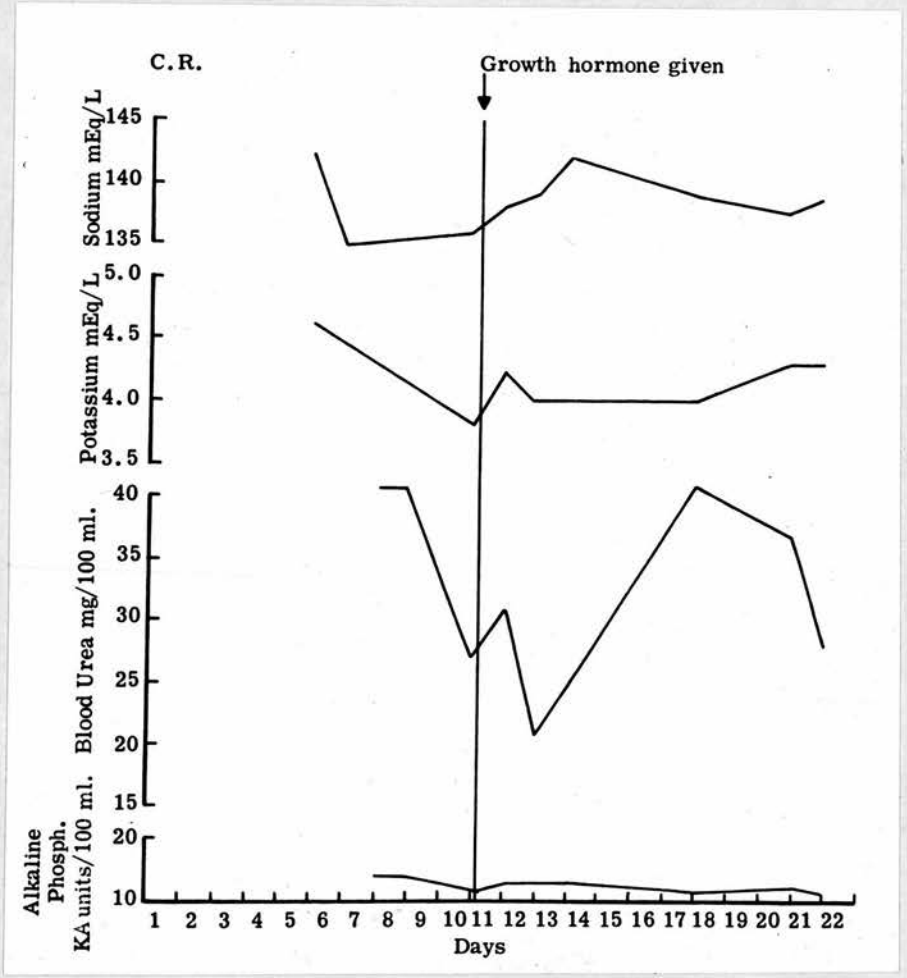

Fig. 12.

PATIENT NO. 12 (M.F., MALE)

NITROGEN BALANCE

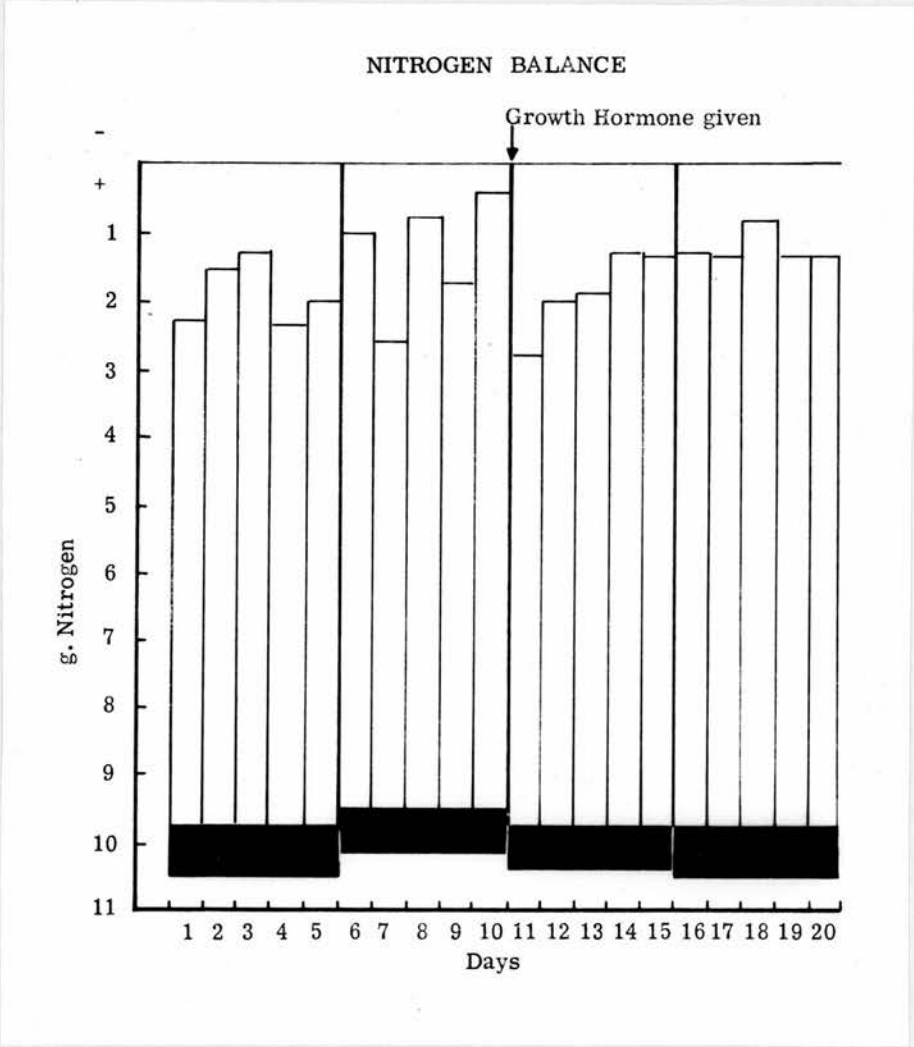

Fig. 13.

Table 27.

NITROGEN (g.)

PATIENT NO. 12 (M.F., MALE)

Period:	1	2	3	4
Dates:	10-15.2.61	15-20.2.61	20-25.2.61	25.2.61-2.3.61
Diet	56.3	55.1	56.1	55.9
Rejects	3.8	3.8	4.2	3.1
Intake	52.5	51.3	51.9	52.8
Urine	39.0	41.1	39.3	42.6
Faeces	4.1	3.6	3.7	4.0
Output	43.1	44.7	43.0	46.6
Balance/5 days	+9.4	+6.6	+8.9	+6.2
Balance/1 day	+1.9	+1.3	+1.8	+1.2

Daily urinary output:

Period No.	Date	Nitrogen (g.)
1	10-11.2.61.	7.5
	11-12.2.61.	8.3
	12-13.2.61.	8.6
	13-14.2.61.	6.9
	14-15.2.61.	7.7
2	15-16.2.61.	8.5
	16-17.2.61.	6.9
	17-18.2.61.	8.8
	18-19.2.61.	7.8
	19-20.2.61.	9.1
3	20-21.2.61.	6.9
	21-22.2.61.	7.7
	22-23.2.61.	7.8
	23-24.2.61.	8.5
	24-25.2.61.	8.4
4	25-26.2.61.	8.5
	26-27.2.61.	8.4
	27-28.2.61.	8.9
	28.2.61. - 1.3.61.	8.4
	1-2.3.61.	8.4

PATIENT NO. 24 (C.L., MALE)

NITROGEN BALANCE

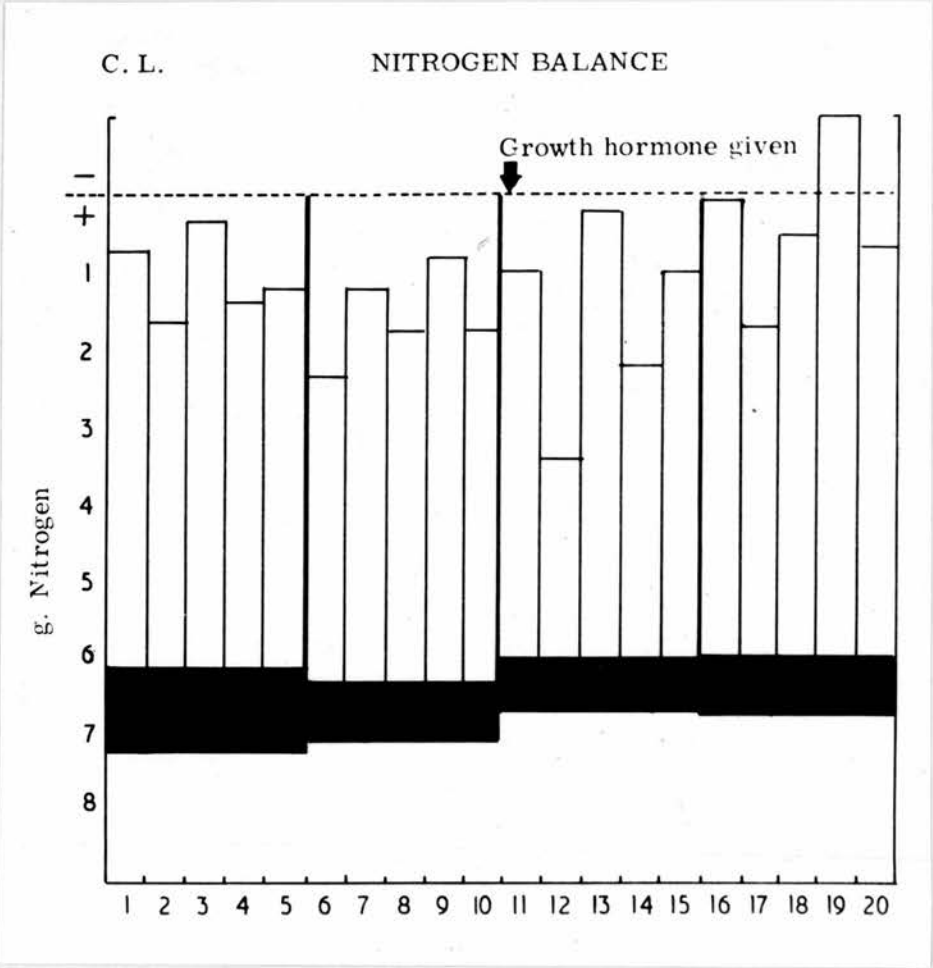

Fig. 14.

Table 28.

NITROGEN (g.)

PATIENT NO. 24 (C.L., MALE)

Period:	1	2	3	4
Dates:	26-31.3.60	31.3.60-5.4.60	5-10.4.60	10-15.4.60
Diet	36.70	36.8	34.0	34.2
Reject	0.12	0.3	nil	nil
Intake	36.60	35.5	34.0	34.2
Urine	25.5	23.9	22.6	28.3
Faeces	5.1	3.6	3.4	4.1
Output	30.6	27.5	26.0	32.4
Balance/5 days	+6	+8	+8	+1.8
Balance/1 day	+1.2	+1.6	+1.6	+0.4

Daily urinary output:

Period No.	Date	Nitrogen (g.)
1	26-27.3.60.	5.50
	27-28.3.60.	4.55
	28-29.3.60.	5.80
	29-30.3.60.	4.75
	30-31.3.60.	4.95
2	31-1.4.60.	4.00
	1-2.4.60.	5.20
	2-3.4.60.	4.60
	3-4.4.60.	5.55
	4-5.4.60.	4.58
3	5-6.4.60.	5.10
	6-7.4.60.	2.69
	7-8.4.60.	5.90
	8-9.4.60.	3.91
	9-10.4.60.	5.03
4	10-11.4.60.	6.05
	11-12.4.60.	4.45
	12-13.4.60.	5.55
	13-14.4.60.	6.85
	14-15.4.60.	5.39

PATIENT NO. 31 (D.R., MALE)

NITROGEN BALANCE

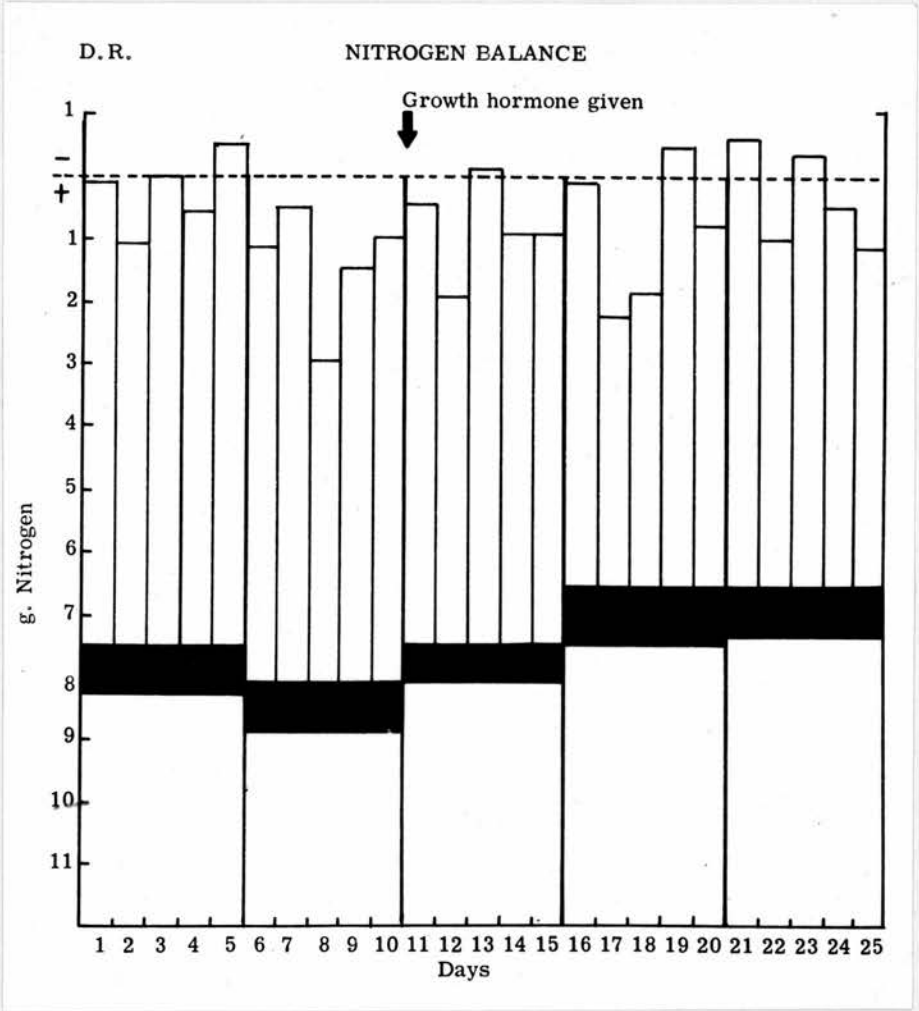

Fig. 15.

Table 29.

NITROGEN (g.)

PATIENT NO. 31 (D.R., MALE)

Period:	1	2	3	4	5
Dates:	2-7.11.61	7-12.11.61	12-17.11.61	17-22.11.61	22-27.11.61
Diet	44.78	45.88	42.58	45.38	44.34
Reject	3.30	1.40	1.50	7.90	7.70
Intake	41.50	44.50	41.10	37.50	36.60
Urine	35.7	33.6	33.8	28.3	31.0
Faeces	4.2	3.8	3.8	4.7	3.8
Output	39.9	37.4	37.6	33.0	34.8
Balance/5 days	+1.6	+7.1	+3.5	+4.5	+1.8
Balance/1 day	+0.3	+1.4	+0.7	+0.9	+0.4

Daily urinary output:

Period No.	Date	Nitrogen (g.)
1	2-3.11.61.	7.3
	3-4.11.61.	6.3
	4-5.11.61.	7.4
	5-6.11.61.	6.8
	6-7.11.61.	8.0
2	7-8.11.61.	6.9
	8-9.11.61.	7.8
	9-10.11.61.	5.2
	10-11.11.61.	6.7
	11-12.11.61.	7.1
3	12-13.11.61.	7.1
	13-14.11.61.	5.6
	14-15.11.61.	7.7
	15-16.11.61.)	13.4
	16-17.11.61.)	
4	17-18.11.61.	6.5
	18-19.11.61.	4.3
	19-20.11.61.	4.7
	20-21.11.61.	7.0
	21-22.11.61.	5.8
5	22-23.11.61.	7.2
	23-24.11.61.	5.6
	24-25.11.61.	6.9
	25-26.11.61.	6.1
	26-27.11.61.	5.4

PATIENT NO. 33 (C.R., MALE)

NITROGEN BALANCE

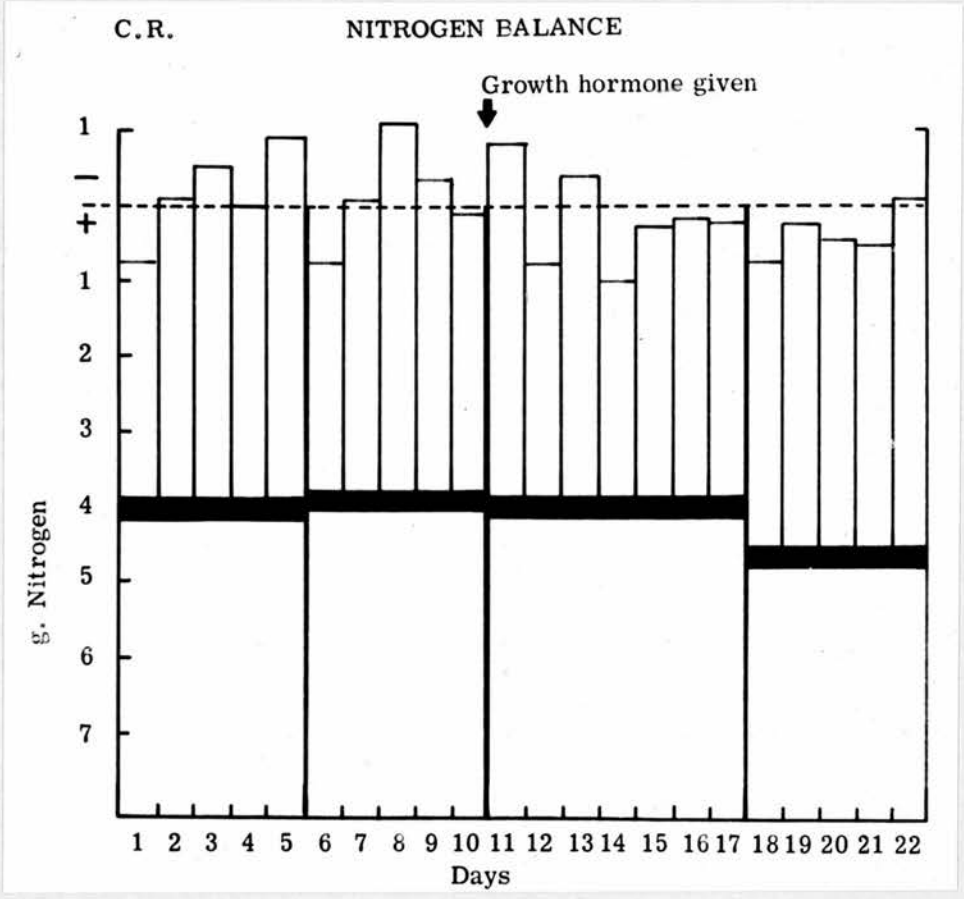

Fig. 16.

Table 30.

NITROGEN (g.)

PATIENT NO. 33 (C.R., MALE)

Period:	1	2	3	4
Dates:	16-21.6.61	21-26.6.61	26-3.7.61	3-8.7.61
Diet	24.1	23.7	32.3	25.4
Reject	3.2	3.4	3.2	1.4
Intake	20.9	20.3	29.1	24.0
Urine	20.0	19.1	22.0	20.4
Faeces	1.7	2.1	2.5	1.6
Output	21.7	21.2	24.5	22.0
Balance/5 days	-0.8	-0.9	+5.6(7 days)	+2.0
Balance/1 day	-0.2	-0.2	+0.8	+0.4

Daily urinary output:

Period No.	Date	Nitrogen (g.)
1	16-17.6.61.	3.1
	17-18.6.61.	4.0
	18-19.6.61.	4.3
	19-20.6.61.	3.9
	20-21.6.61.	4.7
2	21-22.6.61.	2.9
	22-23.6.61.	3.8
	23-24.6.61.	4.8
	24-25.6.61.	4.0
	25-26.6.61.	3.6
3	26-27.6.61.	4.6
	27-28.6.61.	3.1
	28-29.6.61.	4.1
	29-30.6.61.	2.9
	30-1.7.61.	3.5
	1-2.7.61.	3.7
	2-3.7.61.	3.6
4	3-4.7.61.	3.8
	4-5.7.61.	4.2
	5-6.7.61.	4.0
	6-7.7.61.	3.9
	7-8.7.61.	4.6

PATIENT NO. 12 (M.F., MALE)

CALCIUM BALANCE

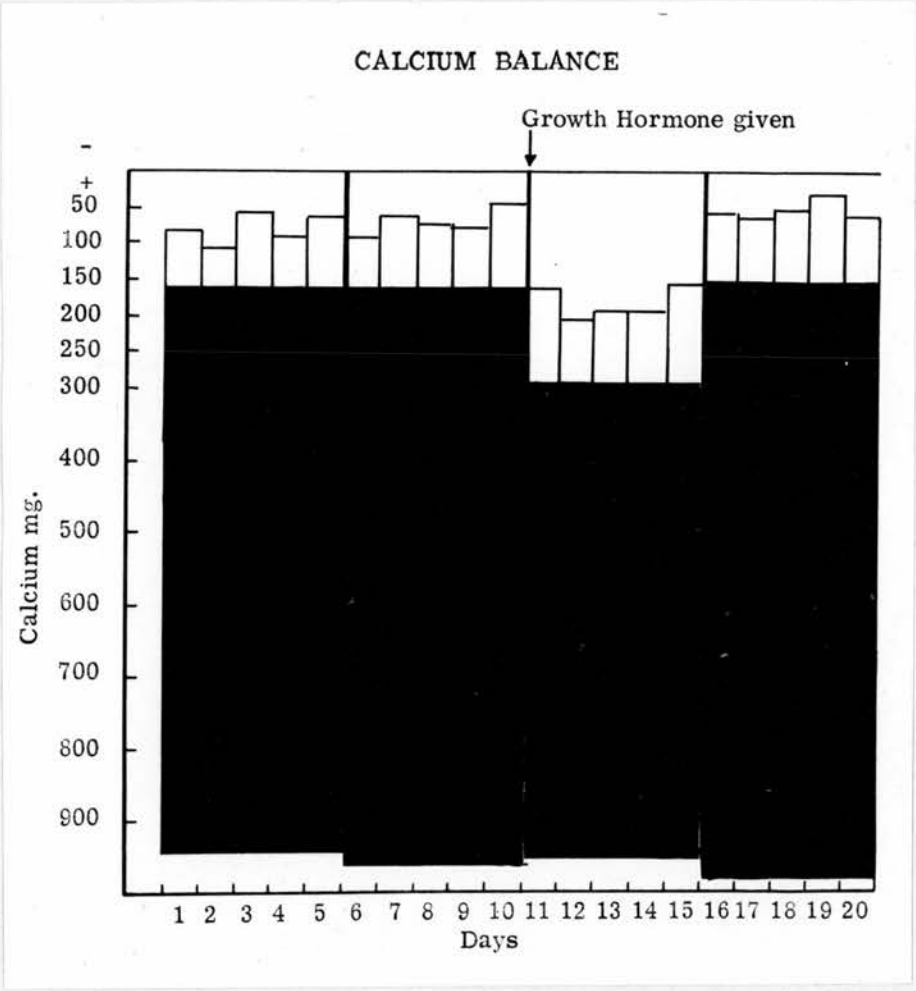

Fig. 17.

Table 31.

CALCIUM (mg.)

PATIENT NO. 12 (M.F., MALE)

Period:	1	2	3	4
Dates:	10-15.2.61	15-20.2.61	20-25.2.61	25-2.3.61
Diet	5238.7	4913.4	5093.4	4954.6
Reject	515.6	69.2	307.7	17.6
Intake	4723.1	4844.2	4785.7	4937.0
Urine	442.0	491.5	498.6	509.5
Faeces	3882.1	4024.4	3365.4	4166.7
Output	4324.1	4515.9	3864.0	4676.2
Balance/5 days	+399.0	+328.3	+921.7	+261.8
Balance/1 day	+79.8	+65.7	+184.3	+52.4

Daily urinary output:

Period No.	Date	Calcium (mg.)
1	10-11.2.61.	88.8
	11-12.2.61.	59.2
	12-13.2.61.	109.4
	13-14.2.61.	78.4
	14-15.2.61.	106.2
2	15-16.2.61.	80.5
	16-17.2.61.	102.4
	17-18.2.61.	95.4
	18-19.2.61.	92.2
	19-20.2.61.	121.0
3	20-21.2.61.	116.0
	21-22.2.61.	79.5
	22-23.2.61.	88.8
	23-24.2.61.	90.0
	24-25.2.61.	124.3
4	25-26.2.61.	95.0
	26-27.2.61.	94.0
	27-28.2.61.	105.0
	28-1.3.61.	121.7
	1-2.3.61.	93.8

PATIENT NO. 24 (C.L., MALE)

CALCIUM BALANCE

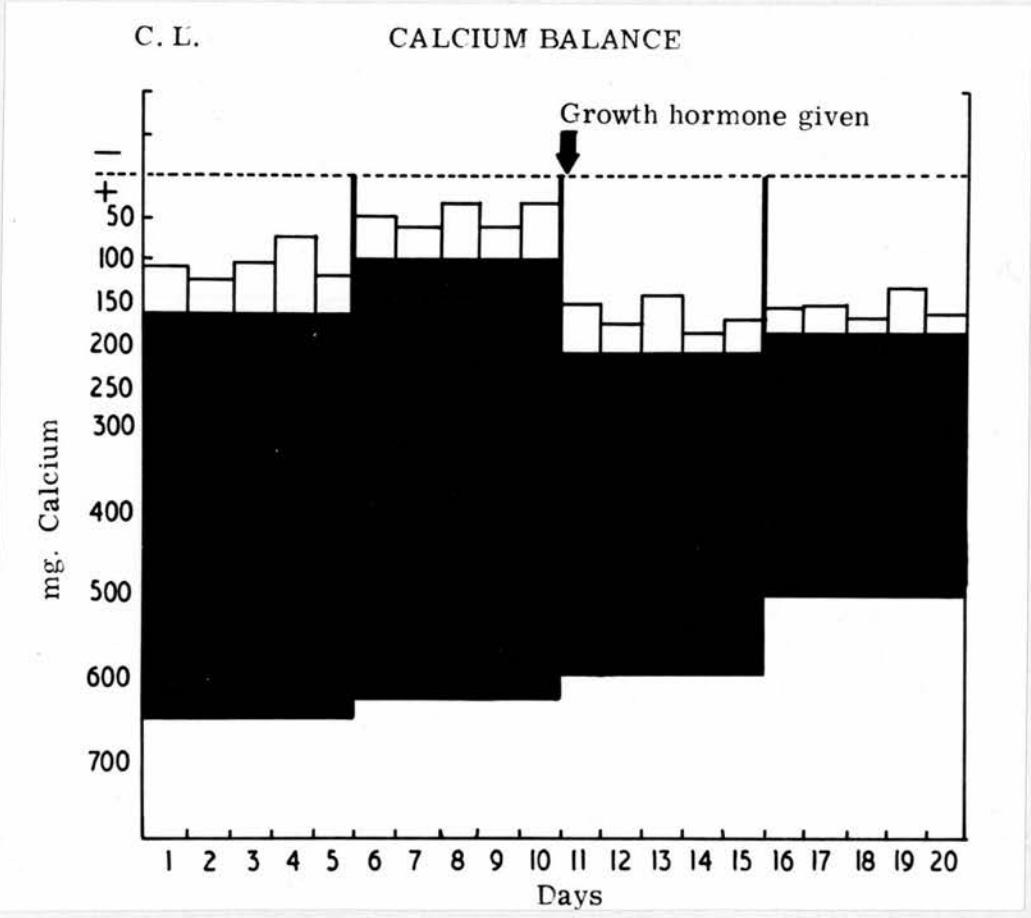

Fig. 18.

Table 32.

CALCIUM (mg.)

PATIENT NO. 24 (C.L., MALE)

Period:	1	2	3	4
Dates:	26-31.3.60	31-5.4.60	5-10.4.60	10-15.4.60
Diet	3240	3150	3000	2520
Reject	nil	nil	nil	nil
Intake	3240	3150	3000	2520
Urine	276	228	212	136
Faeces	2420	2670	1950	1600
Output	2696	2898	2162	1736
Balance/5 days	+544	+252	+838	+784
Balance/1 day	+109	+50	+168	+157

Daily urinary output:

Period No.	Date	Calcium (mg.)
1	26-27.3.60.	54.7
	27-28.3.60.	39.8
	28-29.3.60.	59.5
	29-30.3.60.	79.5
	30-31.3.60.	42.6
2	31-1.4.60.	45.5
	1-2.4.60.	36.0
	2-3.4.60.	55.0
	3-4.4.60.	37.0
	4-5.4.60.	54.0
3	5-6.4.60.	57.8
	6-7.4.60.	32.3
	7-8.4.60.	52.0
	8-9.4.60.	21.8
	9-10.4.60.	38.6
4	10-11.4.60.	26.2
	11-12.4.60.	26.8
	12-13.4.60.	17.8
	13-14.4.60.	45.0
	14-15.4.60.	20.4

PATIENT NO. 31 (D.R., MALE)

CALCIUM BALANCE

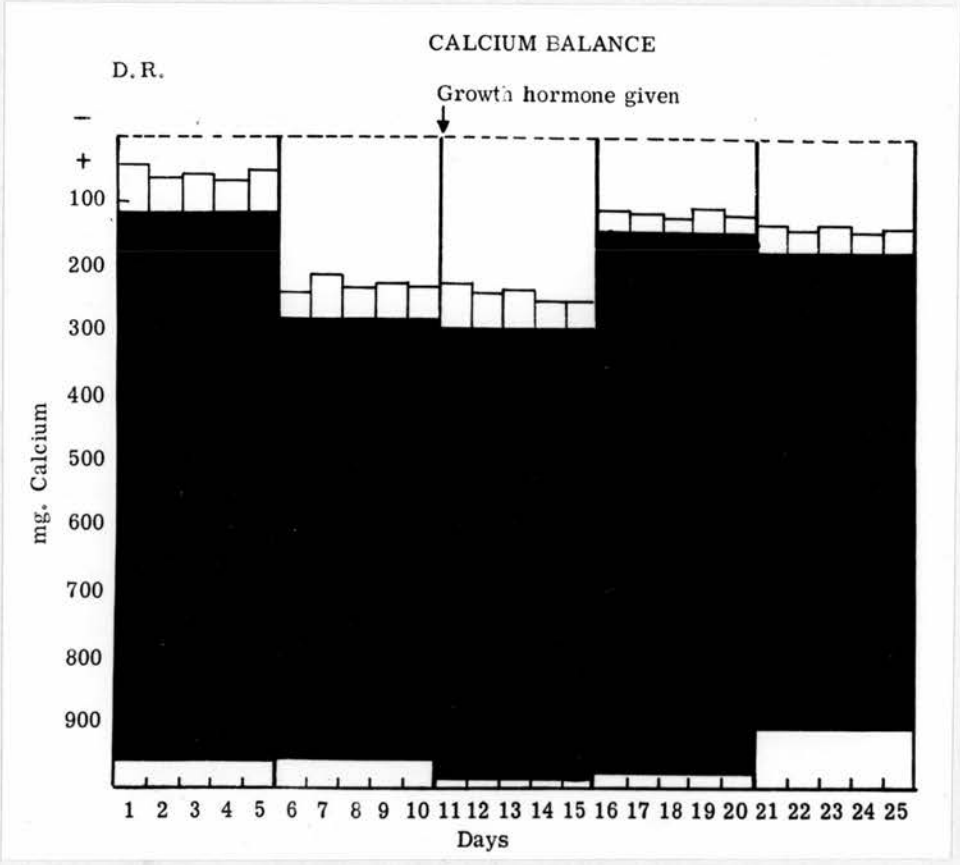

Fig. 19.

Table 33.

CALCIUM (mg.)

PATIENT NO. 31 (D.R., MALE)

Period:	1	2	3	4	5
Dates:	2-7.11.61	7-12.11.61	12-17.11.61	17-22.11.61	22-27.11.61
Diet	5028	4914	5080	5090	4860
Reject	200	116	140	200	340
Intake	4828	4789	4940	4890	4520
Urine	296	263	274	123	164
Faeces	4200	3400	3460	4180	3660
Output	4496	3663	3734	4303	3824
Balance/5 days	+332	+1135	+1206	+587	+696
Balance/1 day	+66	+227	+241	+117	+139

Daily urinary output:

Period No.	Date	Calcium (mg.)
1	2-3.11.61.	68.0
	3-4.11.61.	55.4
	4-5.11.61.	57.2
	5-6.11.61.	48.6
	6-7.11.61.	67.0
2	7-8.11.61.	42.8
	8-9.11.61.	62.4
	9-10.11.61.	50.0
	10-11.11.61.	54.0
	11-12.11.61.	53.6
3	12-13.11.61.	69.2
	13-14.11.61.	55.2
	14-15.11.61.	58.8
	15-16.11.61.)	90.4
	16-17.11.61.)	
4	17-18.11.61.	28.4
	18-19.11.61.	18.2
	19-20.11.61.	15.8
	20-21.11.61.	37.6
	21-22.11.61.	22.8
5	22-23.11.61.	39.2
	23-24.11.61.	27.4
	24-25.11.61.	38.4
	25-26.11.61.	26.0
	26-27.11.61.	33.2

PATIENT NO. 33 (C.R., MALE)

CALCIUM BALANCE

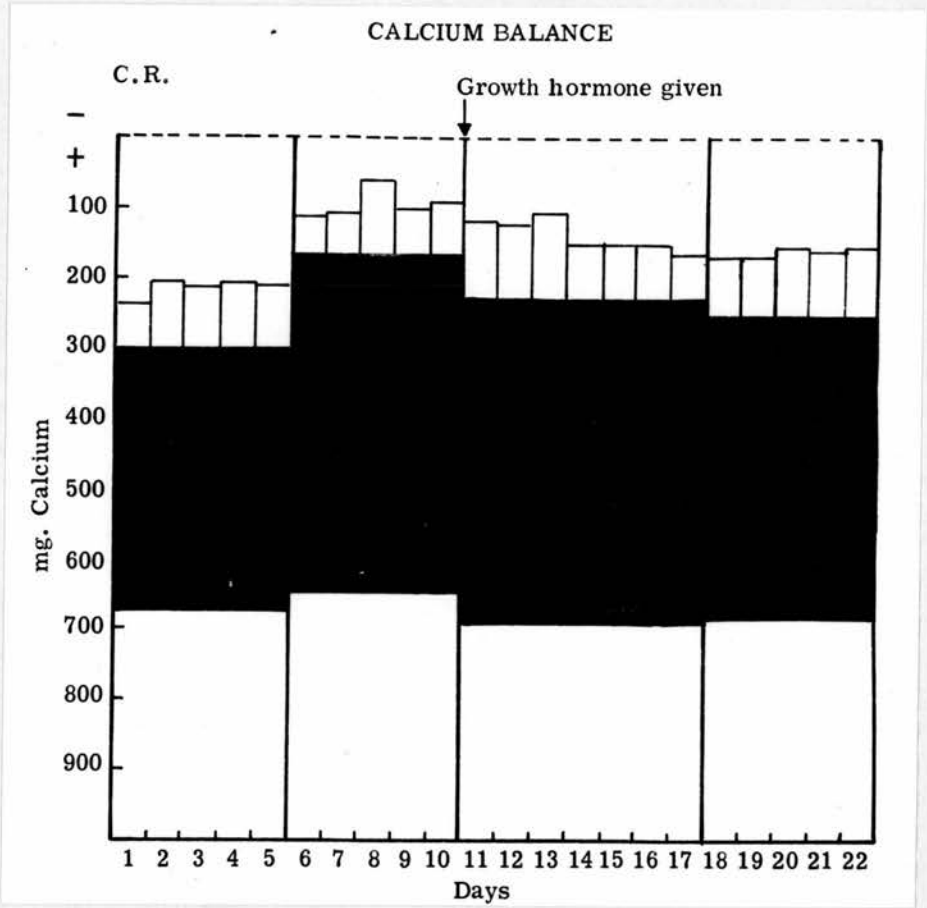

Fig. 20.

Table 34.

CALCIUM (mg.)

PATIENT NO. 33 (C.R., MALE)

Period:	1	2	3	4
Dates:	16-21.6.61	21-26.6.61	26-3.7.61	3-8.7.61
Diet	3659.8	3412.1	5031.1	3630.3
Reject	289.7	178.6	246.0	222.2
Intake	3370.1	3233.5	4785.1	3408.1
Urine	408.0	370.7	631.5	418.3
Faeces	1870.0	2381.0	3199.4	2152.0
Output	2278.0	2751.7	3830.9	2570.3
Balance/5 days	+1092.1	+481.8	+954.2(7 days)	+837.8
Balance/1 day	+218.4	+96.4	+136.3	+167.6

Daily urinary output:

Period No.	Date	Calcium (mg.)
1	16-17.6.61.	64.0
	17-18.6.61.	92.6
	18-19.6.61.	80.1
	19-20.6.61.	86.4
	20-21.6.61.	84.9
2	21-22.6.61.	58.1
	22-23.6.61.	61.8
	23-24.6.61.	107.2
	24-25.6.61.	68.6
	25-26.6.61.	75.0
3	26-27.6.61.	108.8
	27-28.6.61.	100.4
	28-29.6.61.	120.7
	29-30.6.61.	78.6
	30-1.7.61.	79.6
	1-2.7.61.	81.7
	2-3.7.61.	61.7
4	3-4.7.61.	78.9
	4-5.7.61.	77.1
	5-6.7.61.	90.8
	6-7.7.61.	80.3
	7-8.7.61.	91.2

PATIENT NO. 12 (M.F., MALE)

PHOSPHORUS BALANCE

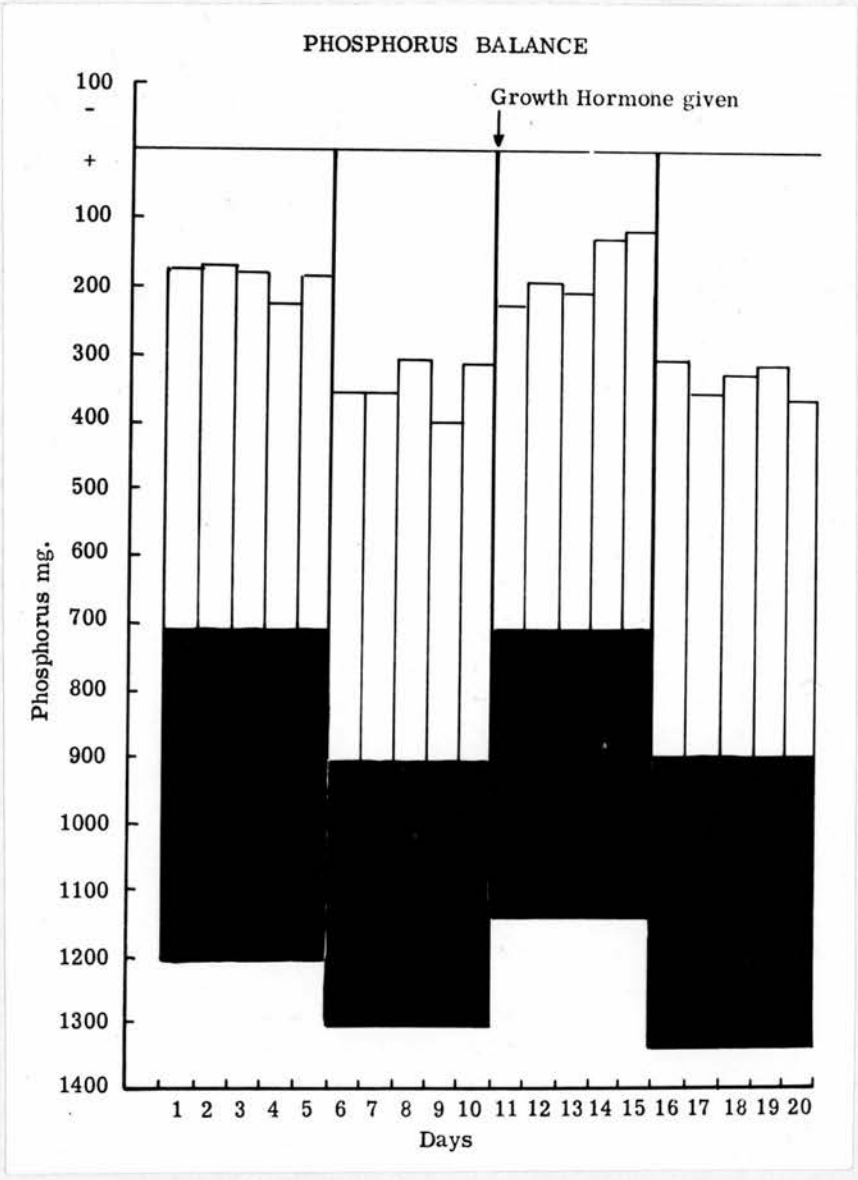

Fig. 21.

Table 35.

PHOSPHORUS (mg.)

PATIENT NO. 12 (M.F., MALE)

Period:	1	2	3	4
Dates:	10-15.2.61	15-20.2.61	20-25.2.61	25-2.3.51
Diet	6513.2	6220.6	6188.3	6458.7
Reject	506.8	320.7	456.1	252.8
Intake	6006.4	6541.3	5732.2	6711.5
Urine	2602.5	2824.9	2681.7	2840.1
Faeces	2500.0	2000.0	2166.7	2192.0
Output	5102.5	4824.9	4848.4	5032.1
Balance/5 days	+903.9	+1716.4	+883.8	+1679.4
Balance/1 day	+180.8	+343.3	+176.8	+335.8

Daily urinary output:

Period No.	Date	Phosphorus (mg.)
1	10-11.2.61.	88.8
	11-12.2.61.	59.2
	12-13.2.61.	109.4
	13-14.2.61.	78.4
	14-15.2.61.	106.2
2	15-16.2.61.	80.5
	16-17.2.61.	102.4
	17-18.2.61.	95.4
	18-19.2.61.	92.2
	19-20.2.61.	121.0
3	20-21.2.61.	116.0
	21-22.2.61.	79.5
	22-23.2.61.	88.8
	23-24.2.61.	90.0
	24-25.2.61.	124.3
4	25-26.2.61.	95.0
	26-27.2.61.	94.0
	27-28.2.61.	105.0
	28-1.3.61.	121.7
	1-2.3.61.	93.8

PATIENT NO. 24 (C.L., MALE)

PHOSPHORUS BALANCE

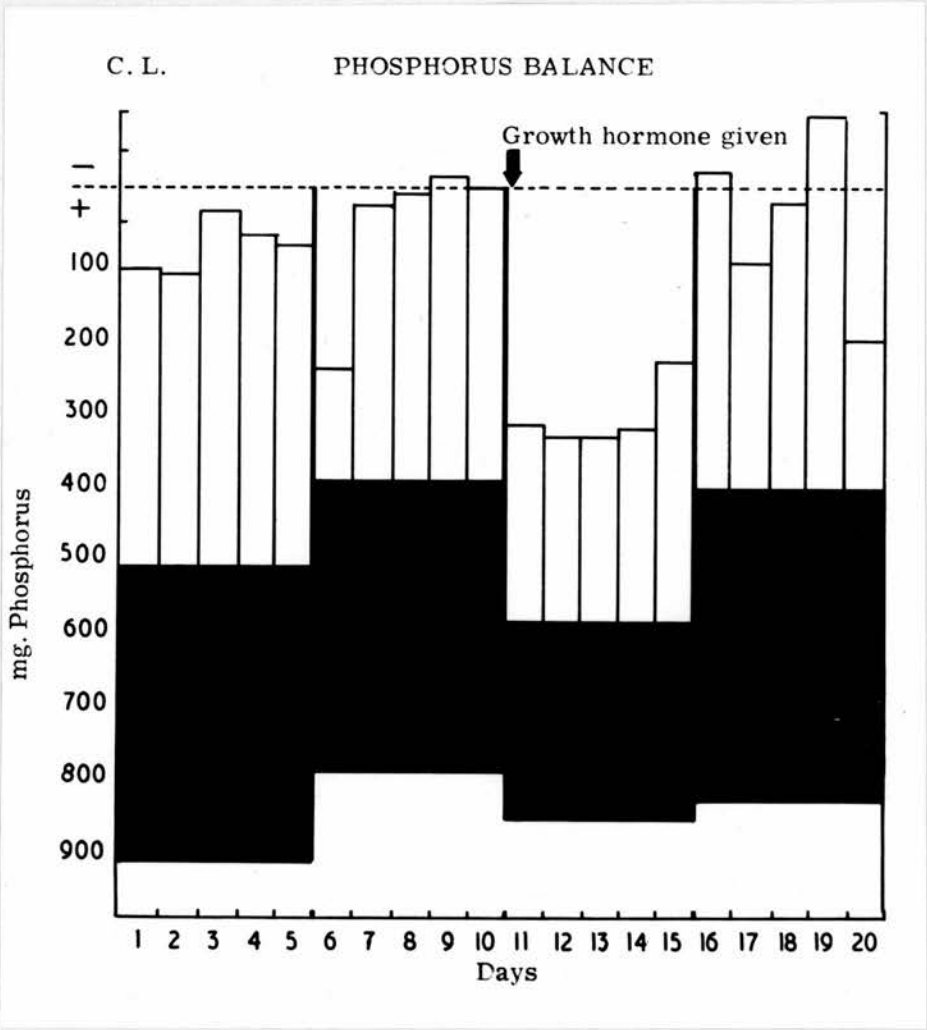

Fig. 22.

Table 36.

PHOSPHORUS (mg.)

PATIENT NO. 24 (C.L., MALE)

Period:	1	2	3	4
Dates:	26-31.3.60	31-5.4.60	5-10.4.60	10-15.4.60
Diet	4600	4000	4300	4250
Reject	nil	18	nil	59
Intake	4600	3982	4300	4191
Urine	2150	1828	1324	1822
Faeces	2030	1970	1350	2150
Output	4180	3798	2674	3972
Balance/5 days	+420	+184	+1626	+219
Balance/1 day	+84	+37	+325	+44

Daily urinary output:

Period No.	Date	Phosphorus (mg.)
1	26-27.3.60.	403
	27-28.3.60.	398
	28-29.3.60.	474
	29-30.3.60.	430
	30-31.3.60.	446
2	31-1.4.60.	248
	1-2.4.60.	380
	2-3.4.60.	390
	3-4.4.60.	410
	4-5.4.60.	400
3	5-6.4.60.	275
	6-7.4.60.	250
	7-8.4.60.	250
	8-9.4.60.	265
	9-10.4.60.	354
4	10-11.4.60.	430
	11-12.4.60.	302
	12-13.4.60.	383
	13-14.4.60.	505
	14-15.4.60.	202

PATIENT NO. 31 (D.R., MALE)

PHOSPHORUS BALANCE

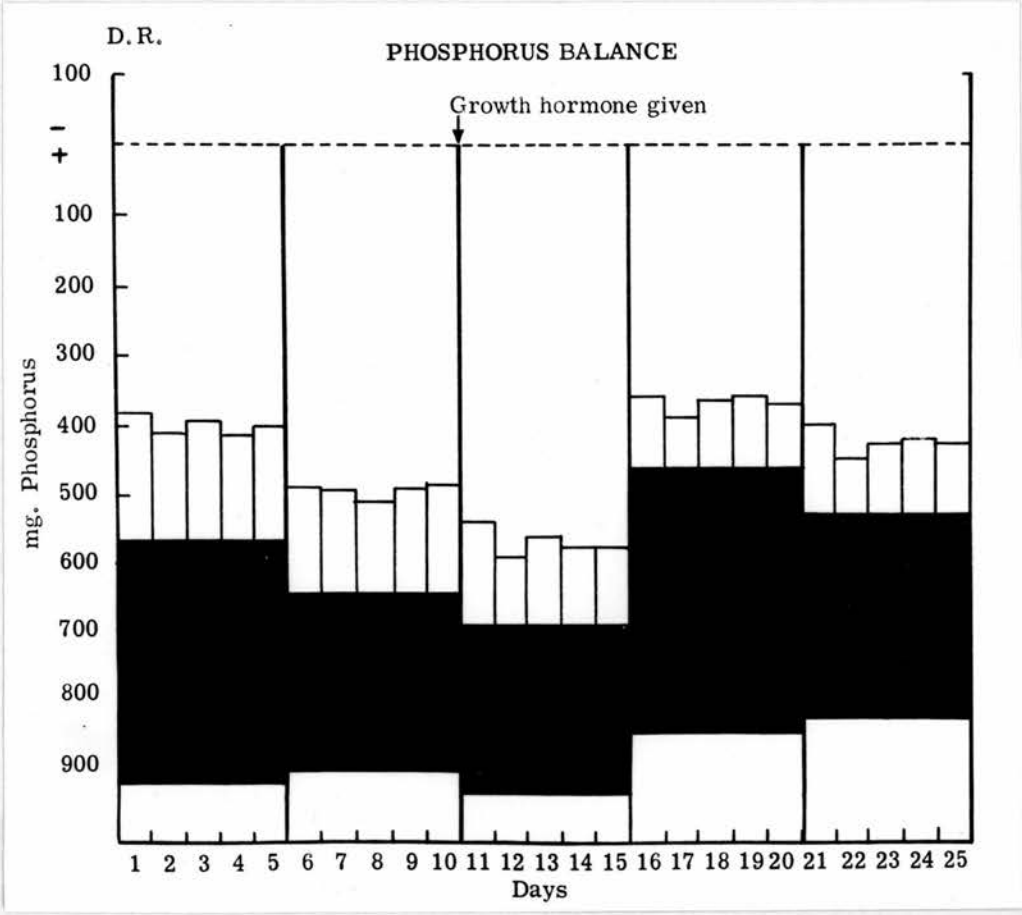

Fig. 23.

PHOSPHORUS (mg.)

PATIENT NO. 31 (D.R., MALE)

Period:	1	2	3	4	5
Dates:	2-7.11.61	7-12.11.61	12-17.11.61	17-22.11.61	22-27.11.61
Diet	4865.2	4692.2	4865.3	4769.1	4711.4
Reject	261.5	188.5	165.3	538.4	596.2
Intake	4603.7	4503.7	4700.0	4230.7	4115.2
Urine	792.8	722.9	602.3	449.3	529.2
Faeces	1802.9	1298.1	1254.8	1898.1	1485.6
Output	2595.7	2021.0	1857.1	2347.4	2014.8
Balance/5 days	+2008.0	+2482.7	+2842.9	+1883.3	+2100.4
Balance/1 day	+401.6	+496.5	+569.0	+376.7	+420.1

Daily urinary output:

Period No.	Date	Phosphorus (mg.)
1	2-3.11.61.	181.5
	3-4.11.61.	148.1
	4-5.11.61.	164.9
	5-6.11.61.	134.9
	6-7.11.61.	163.4
2	7-8.11.61.	152.5
	8-9.11.61.	144.7
	9-10.11.61.	130.3
	10-11.11.61.	146.1
	11-12.11.61.	149.3
3	12-13.11.61.	150.4
	13-14.11.61.	101.0
	14-15.11.61.	128.8
	15-16.11.61.) 16-17.11.61.)	222.1
4	17-18.11.61.	104.0
	18-19.11.61.	71.2
	19-20.11.61.	96.9
	20-21.11.61.	99.4
	21-22.11.61.	77.8
5	22-23.11.61.	134.4
	23-24.11.61.	84.2
	24-25.11.61.	103.9
	25-26.11.61.	102.3
	26-27.11.61.	104.4

PATIENT NO. 33 (C.R., MALE)

PHOSPHORUS BALANCE

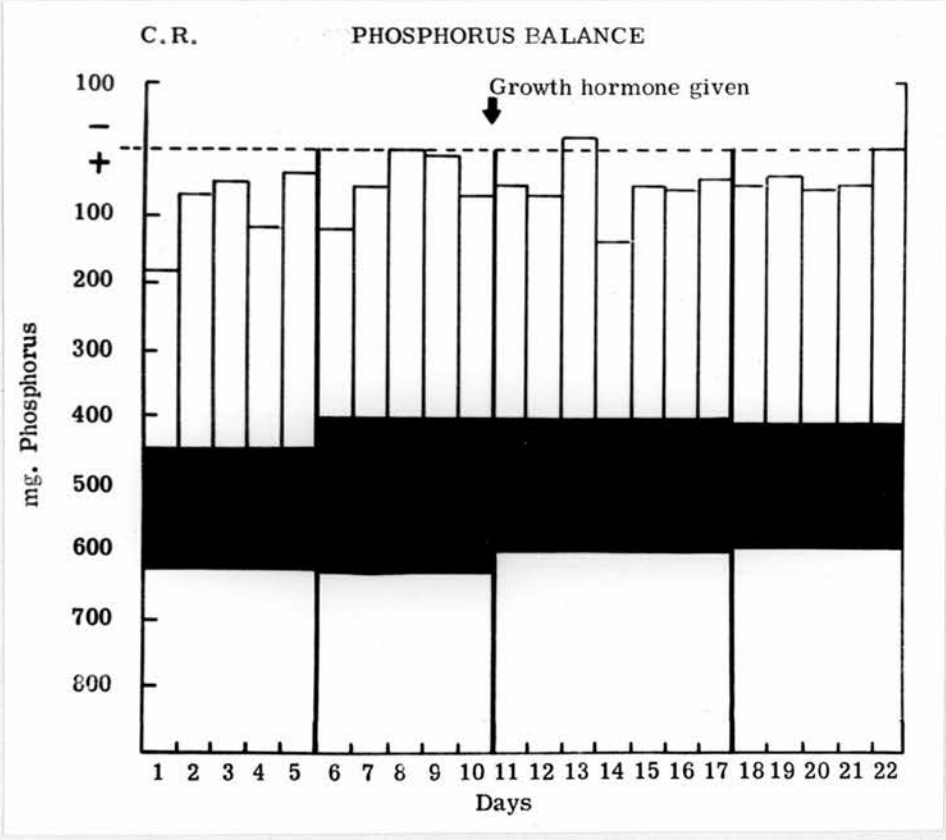

Fig. 24.

Table 38.

PHOSPHORUS (mg.)

PATIENT NO. 33 (C.R., MALE)

Period:	1	2	3	4
Dates:	16-21.6.61	21-26.6.61	26-3.7.61	3-8.7.61
Diet	3482.8	3444.3	4739.0	3299.2
Reject	368.6	264.4	528.8	336.5
Intake	3114.2	3179.9	4210.2	2962.7
Urine	1772.4	1739.5	2438.9	1862.9
Faeces	881.4	1141.0	1356.2	876.7
Output	2653.8	2880.5	3795.1	2739.6
Balance/5 days	+460.4	+299.4	+415.1 (7 days)	+223.1
Balance/1 day	+92.1	+59.9	+59.3	+44.8

Daily urinary output:

Period No.	Date	Phosphorus (mg.)
1	16-17.6.61.	265.1
	17-18.6.61.	372.9
	18-19.6.61.	395.7
	19-20.6.61.	328.4
	20-21.6.61.	410.3
2	21-22.6.61.	278.6
	22-23.6.61.	346.3
	23-24.6.61.	403.4
	24-25.6.61.	387.2
	25-26.6.61.	324.0
3	26-27.6.61.	351.7
	27-28.6.61.	330.0
	28-29.6.61.	431.1
	29-30.6.61.	270.0
	30-1.7.61.	350.5
	1-2.7.61.	345.6
	2-3.7.61.	360.0
4	3-4.7.61.	358.6
	4-5.7.61.	371.0
	5-6.7.61.	357.9
	6-7.7.61.	358.4
	7-8.7.61.	417.0

PATIENT NO. 12 (M.F., MALE)

SODIUM BALANCE

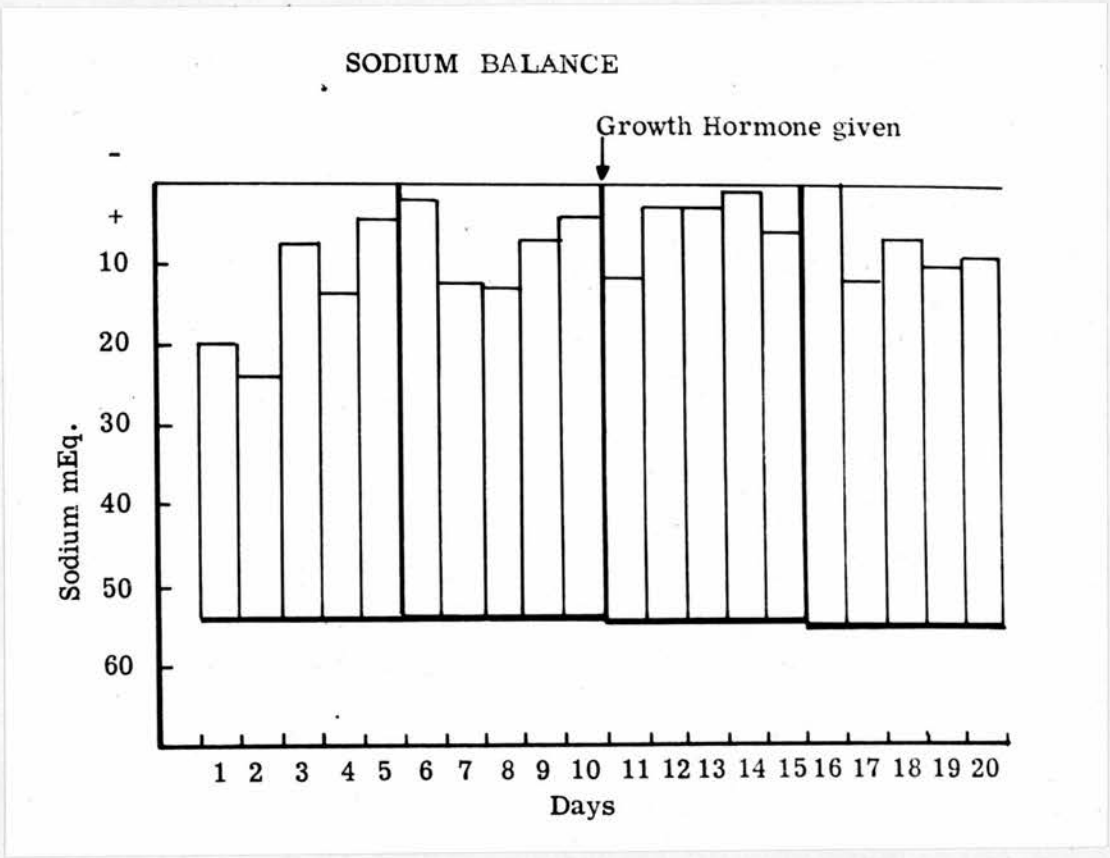

Fig. 25.

SODIUM (m.Eq.)

PATIENT NO. 12 (M.F., MALE)

Period:	1	2	3	4
Dates:	10-15.2.61	15-20.2.61	20-25.2.61	25-2.3.61
Diet	287.3	283.8	290.5	287.1
Reject	16.8	14.2	16.5	7.1
Intake	270.5	269.6	274.0	280.0
Urine	198.2	226.1	244.5	238.2
Faeces	1.2	0.9	1.3	1.8
Output	199.4	227.0	245.8	240.0
Balance/5 days	+71.1	+42.6	+28.2	+40.0
Balance/1 day	+14.2	+8.5	+5.6	+8.0

Daily urinary output:

Period No.	Date	Sodium (m.Eq.)
1	10-11.2.61.	33.5
	11-12.2.61.	29.9
	12-13.2.61.	46.2
	13-14.2.61.	40.0
	14-15.2.61.	48.6
2	15-16.2.61.	51.0
	16-17.2.61.	40.4
	17-18.2.61.	38.9
	18-19.2.61.	46.2
	19-20.2.61.	49.6
3	20-21.2.61.	42.1
	21-22.2.61.	50.9
	22-23.2.61.	50.5
	23-24.2.61.	52.8
	24-25.2.61.	48.2
4	25-26.2.61.	55.5
	26-27.2.61.	42.8
	27-28.2.61.	48.4
	28-1.3.61.	45.1
	1-2.3.61.	46.4

PATIENT NO. 24 (C.L., MALE)

SODIUM BALANCE

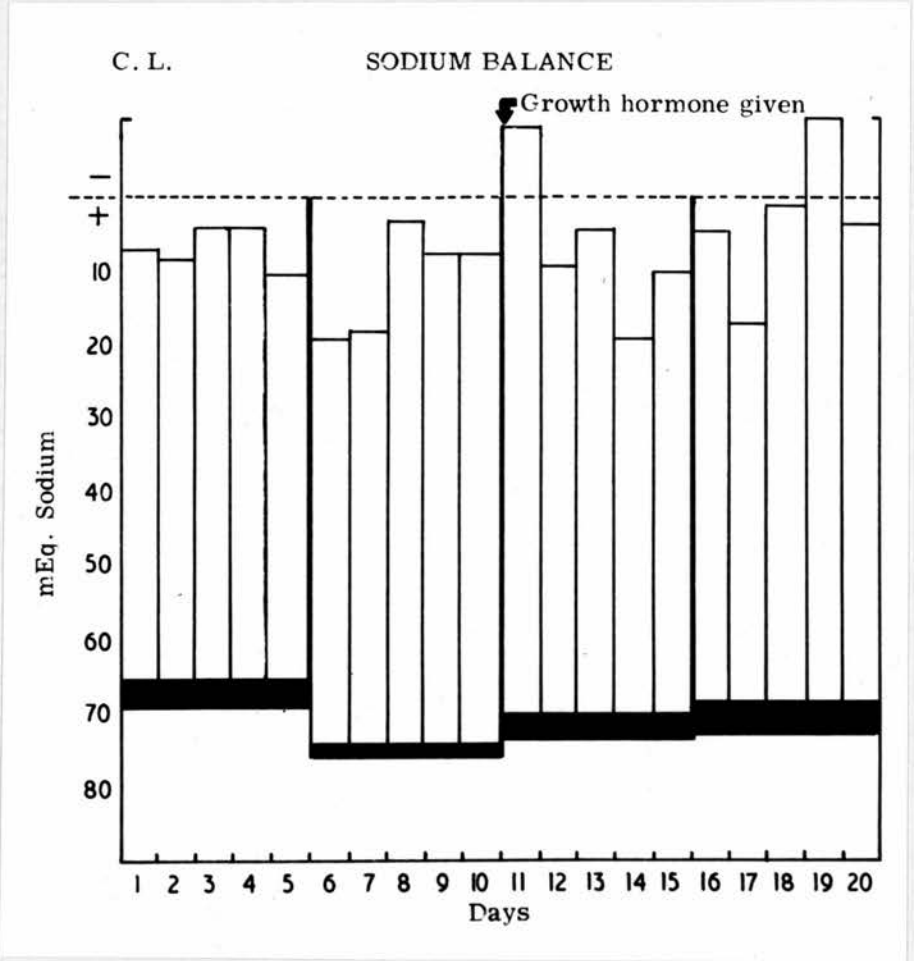

Fig. 26.

Table 40.

SODIUM (m.Eq.)

PATIENT NO. 24 (C.L., MALE)

Period:	1	2	3	4
Dates:	26-31.3.60	31-5.4.60	5-10.4.60	10-15.4.60
Diet	342.0	387.0	369.0	369.0
Reject	0.9	7.2	nil	4.5
Intake	341.1	379.8	369.0	364.5
Urine	293	320.1	288.5	331
Faeces	20	8.0	17.4	18
Output	313	328.1	305.9	349
Balance/5 days	+28.1	+51.7	+53.1	+15.5
Balance/1 day	+5.6	+10.3	+10.6	+3.1

Daily urinary output:

Period No.	Date	Sodium (m.Eq.)
1	26-27.3.60.	58.5
	27-28.3.60.	57.5
	28-29.3.60.	61.0
	29-30.3.60.	61.1
	30-31.3.60.	54.9
2	31-1.4.60.	56.3
	1-2.4.60.	57.3
	2-3.4.60.	72.5
	3-4.4.60.	67.0
	4-5.4.60.	67.0
3	5-6.4.60.	79.5
	6-7.4.60.	51.5
	7-8.4.60.	56.5
	8-9.4.60.	46.5
	9-10.4.60.	55.5
4	10-11.4.60.	64.5
	11-12.4.60.	51.5
	12-13.4.60.	69.0
	13-14.4.60.	80.5
	14-15.4.60.	65.5

PATIENT NO. 31 (D.R., MALE)

SODIUM BALANCE

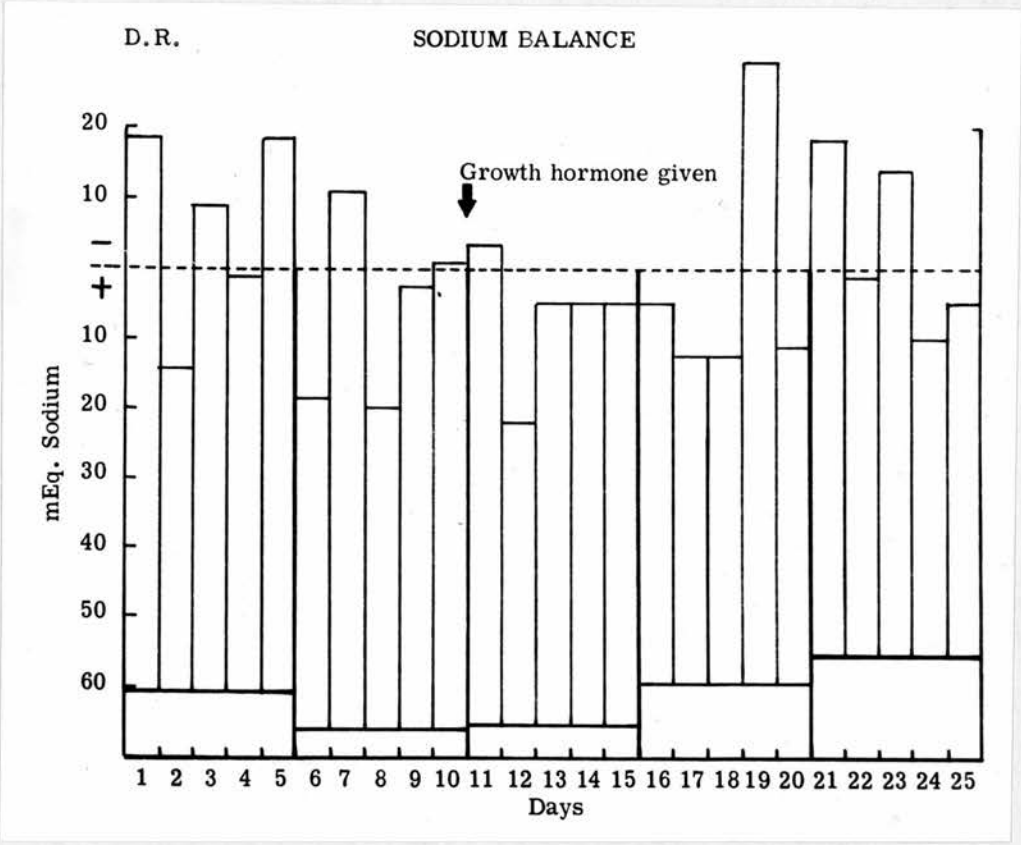

Fig. 27.

Table 41.

SODIUM (m.Eq.)

PATIENT NO. 31 (D.R., MALE)

Period:	1	2	3	4	5
Dates:	2-7.11.61	7-12.11.61	12-17.11.61	17-22.11.61	22-27.11.61
Diet	341.0	341.0	338.5	343.5	333.5
Reject	33.8	10.0	12.5	47.5	58.8
Intake	307.2	331.0	326.0	296.0	274.7
Urine	333.1	300.6	289.1	282.4	288.0
Faeces	1.4	1.6	2.6	2.9	1.4
Output	334.5	302.2	291.7	285.3	289.4
Balance/5 days	-27.3	+28.8	+34.3	+10.7	-15.7
Balance/1 day	-5.5	+5.8	+6.8	+2.1	-3.1

Daily urinary output:

Period No.	Date	Sodium (m.Eq.)
1	2-3.11.61.	79.3
	3-4.11.61.	46.6
	4-5.11.61.	69.2
	5-6.11.61.	59.7
	6-7.11.61.	78.3
2	7-8.11.61.	47.6
	8-9.11.61.	77.0
	9-10.11.61.	45.5
	10-11.11.61.	63.6
	11-12.11.61.	66.9
3	12-13.11.61.	67.2
	13-14.11.61.	41.7
	14-15.11.61.	59.2
	15-16.11.61.)	121.0
	16-17.11.61.)	
4	17-18.11.61.	53.0
	18-19.11.61.	46.0
	19-20.11.61.	45.9
	20-21.11.61.	89.8
	21-22.11.61.	47.7
5	22-23.11.61.	72.7
	23-24.11.61.	53.8
	24-25.11.61.	68.4
	25-26.11.61.	44.1
	26-27.11.61.	49.0

PATIENT NO. 33 (C.R., MALE)

SODIUM BALANCE

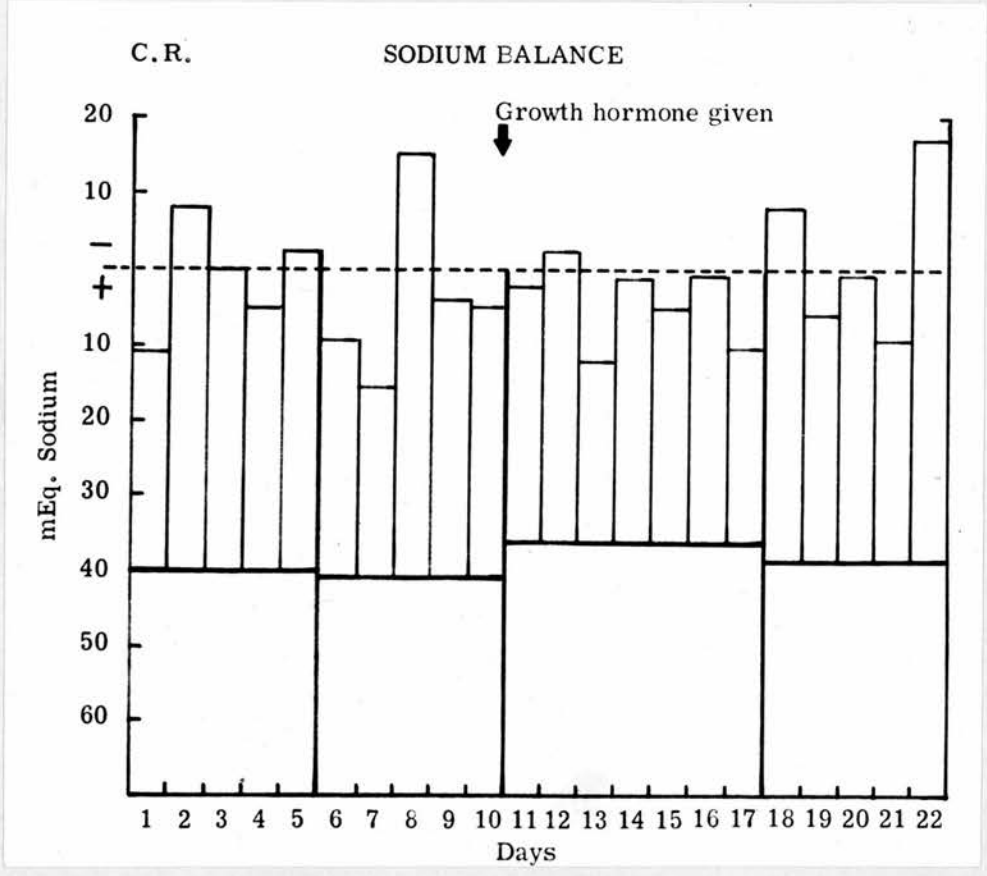

Fig. 28.

Table 42.

SODIUM (m.Eq.)

PATIENT NO. 33 (C.R., MALE)

Period:	1	2	3	4
Dates:	16-21.6.61.	21-26.6.61	26-3.7.61	3-8.7.61
Diet	225.8	226.1	316.4	223.5
Reject	26.2	18.7	60.0	31.2
Intake	199.6	207.4	256.4	192.3
Urine	190.9	186.0	223.1	200.7
Faeces	1.7	2.1	1.2	1.1
Output	192.6	188.1	224.3	201.8
Balance/5 days	+7.0	+19.3	+32.1(7 days)	-9.5
Balance/1 day	+1.4	+4.8	+4.6	-1.9

Daily urinary output:

Period No.	Date	Sodium (m.Eq.)
1	16-17.6.61.	28.2
	17-18.6.61.	47.1
	18-19.6.61.	39.5
	19-20.6.61.	34.4
	20-21.6.61.	41.7
2	21-22.6.61.	31.9
	22-23.6.61.	25.1
	23-24.6.61.	56.0
	24-25.6.61.	37.0
	25-26.6.61.	36.0
3	26-27.6.61.	34.0
	27-28.6.61.	38.0
	28-29.6.61.	23.1
	29-30.6.61.	35.5
	30-1.7.61.	31.0
	1-2.7.61.	35.1
	2-3.7.61.	26.4
4	3-4.7.61.	46.4
	4-5.7.61.	31.7
	5-6.7.61.	37.8
	6-7.7.61.	29.4
	7-8.7.61.	55.4

PATIENT NO. 12 (M.F., MALE)

POTASSIUM BALANCE

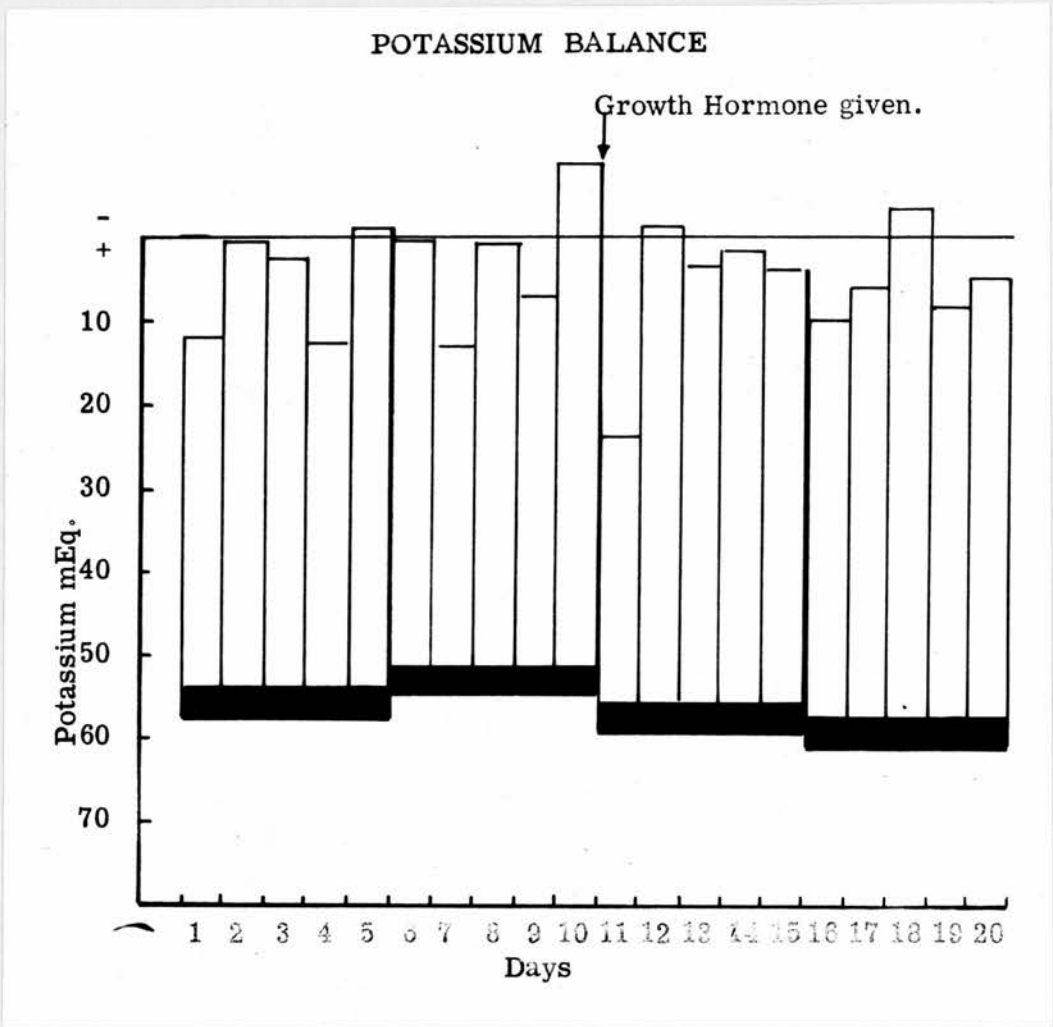

Fig. 29.

Table 43.

POTASSIUM (m.Eq.)

PATIENT NO. 12 (M.F., MALE)

Period:	1	2	3	4
Dates:	10-15.2.61	15-20.2.61	20-25.2.61	25-2.3.61
Diet	312.8	310.0	319.2	331.8
Reject	19.0	32.5	22.8	18.0
Intake	293.8	277.5	296.4	313.8
Urine	240.6	249.5	249.5	265.9
Faeces	24.0	17.5	18.0	22.5
Output	264.6	267.0	267.5	288.4
Balance/5 days	+29.2	+10.5	+28.9	+25.4
Balance/1 day	+5.8	+2.1	+5.8	+5.1

Daily urinary output:

Period No.	Date	Potassium (m.Eq.)
1	10-11.2.61.	41.0
	11-12.2.61.	53.7
	12-13.2.61.	51.8
	13-14.2.61.	39.8
	14-15.2.61.	54.3
2	15-16.2.61.	52.4
	16-17.2.61.	37.2
	17-18.2.61.	51.0
	18-19.2.61.	45.6
	19-20.2.61.	63.3
3	20-21.2.61.	32.6
	21-22.2.61.	57.2
	22-23.2.61.	52.8
	23-24.2.61.	54.0
	24-25.2.61.	52.9
4	25-26.2.61.	48.3
	26-27.2.61.	52.8
	27-28.2.61.	62.0
	28-1.3.61.	49.6
	1-2.3.61.	53.2

PATIENT NO. 24 (C.L., MALE)

POTASSIUM BALANCE

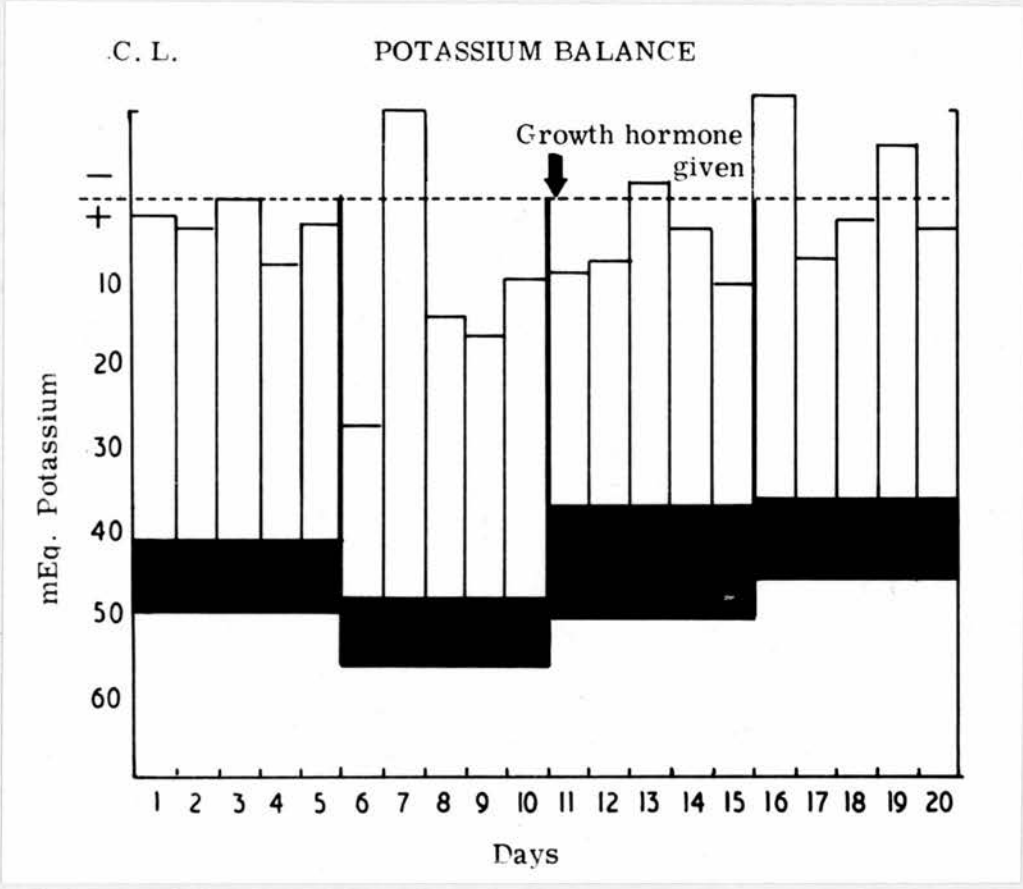

Fig. 30

Table 44.

POTASSIUM (m.Eq.)

PATIENT NO. 24 (C.L., MALE)

Period:	1	2	3	4
Dates:	26-31.3.60	31-5.4.60	5-10.4.60	10-15.4.60
Diet	252.0	283.0	252	234.00
Reject	1.3	1.5	nil	0.75
Intake	250.7	281.5	252	233.20
Urine	188	184.4	156.5	190
Faeces	42	40.0	64.0	48
Output	230	224.4	220.5	238
Balance/5 days	+20	+57.1	+31.5	-5
Balance/1 day	+4	+11.4	+6.3	-1

Daily urinary output:

Period No.	Date	Potassium (m.Eq.)
1	26-27.3.60.	39.6
	27-28.3.60.	37.0
	28-29.3.60.	41.6
	29-30.3.60.	32.6
	30-31.3.60.	37.2
2	31-1.4.60.	20.6
	1-2.4.60.	59.4
	2-3.4.60.	34.0
	3-4.4.60.	31.9
	4-5.4.60.	38.5
3	5-6.4.60.	28.1
	6-7.4.60.	29.0
	7-8.4.60.	38.7
	8-9.4.60.	33.7
	9-10.4.60.	27.0
4	10-11.4.60.	48.0
	11-12.4.60.	30.5
	12-13.4.60.	34.5
	13-14.4.60.	44.0
	14-15.4.60.	34.0

PATIENT NO. 31 (D.R., MALE)

POTASSIUM BALANCE

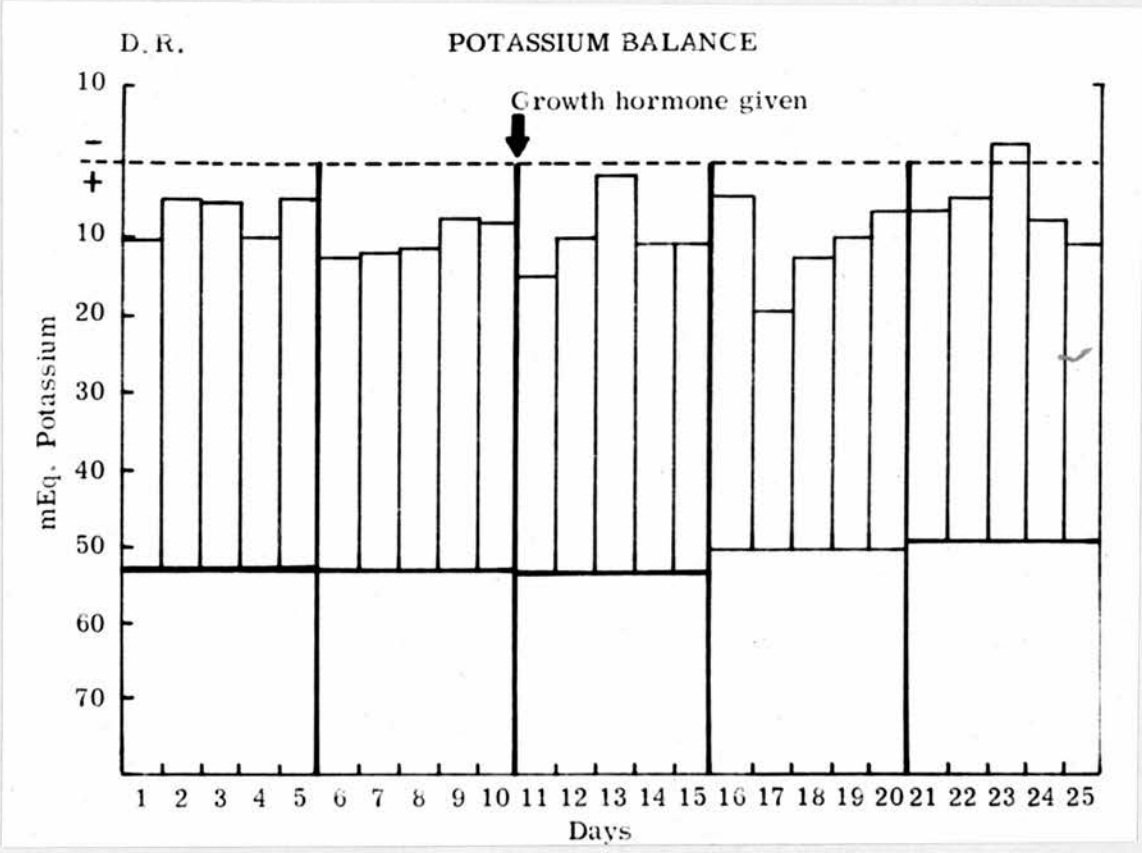

Fig. 31.

Table 45.

POTASSIUM (m.Eq.)

PATIENT NO. 31 (D.R., MALE)

Period:	1	2	3	4	5
Dates:	2-7.11.61	7-12.11.61	12-17.11.61	17-22.11.61	22-27.11.61
Diet	291.1	284.9	283.6	284.9	276.1
Reject	22.5	15.0	10.0	28.8	32.5
Intake	268.6	269.9	273.6	256.1	243.6
Urine	230.3	214.9	221.1	198.8	214.9
Faeces	1.0	0.9	0.9	1.2	0.9
Output	231.3	215.8	222.0	200.0	215.8
Balance/5 days	+37.3	+54.1	+51.6	+56.1	+27.8
Balance/1 day	+7.4	+10.8	+10.3	+11.2	+5.6

Daily urinary output:

Period No.	Date	Potassium (m.Eq.)
1	2-3.11.61.	42.3
	3-4.11.61.	48.6
	4-5.11.61.	47.6
	5-6.11.61.	43.4
	6-7.11.61.	48.4
2	7-8.11.61.	40.3
	8-9.11.61.	40.8
	9-10.11.61.	41.9
	10-11.11.61.	46.4
	11-12.11.61.	45.5
3	12-13.11.61.	38.9
	13-14.11.61.	44.1
	14-15.11.61.	52.4
	15-16.11.61.)	85.7
	16-17.11.61.)	
4	17-18.11.61.	46.4
	18-19.11.61.	31.0
	19-20.11.61.	37.8
	20-21.11.61.	40.0
	21-22.11.61.	43.6
5	22-23.11.61.	41.9
	23-24.11.61.	43.3
	24-25.11.61.	52.4
	25-26.11.61.	40.5
	26-27.11.61.	36.8

PATIENT NO. 33 (C.R., MALE)

POTASSIUM BALANCE

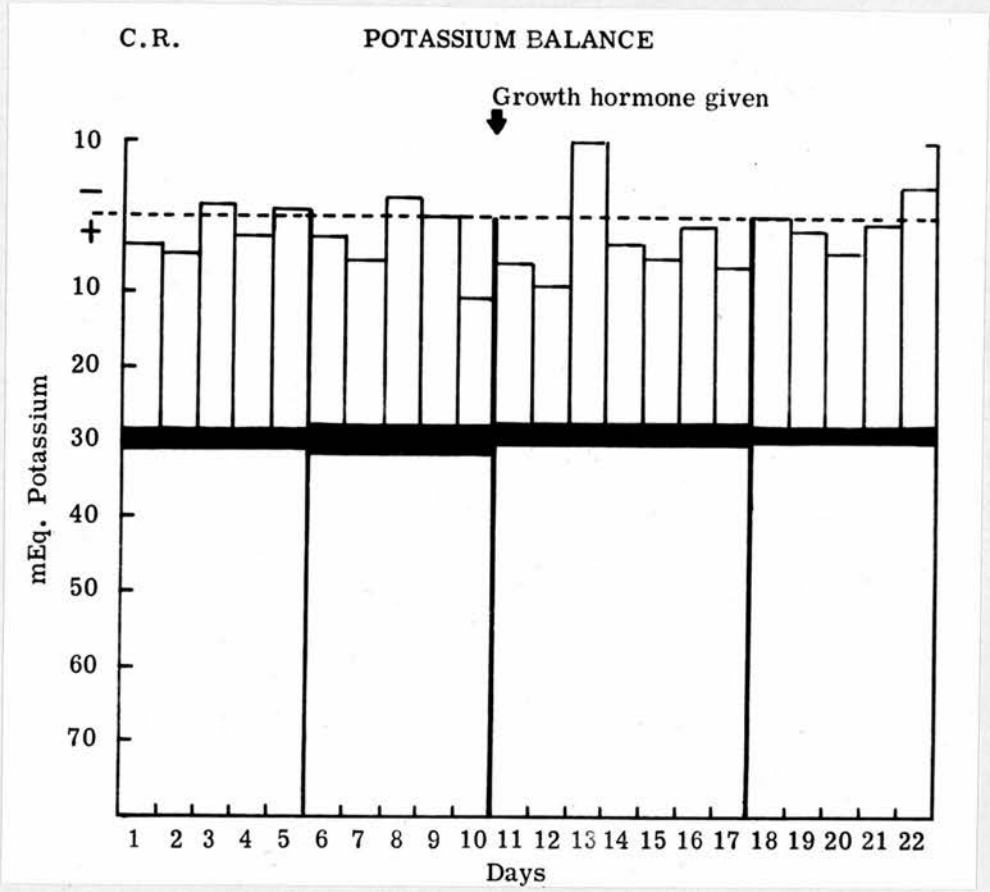

Fig. 32.

Table 46.

POTASSIUM (m.Eq.)

PATIENT NO. 33 (C.R., MALE)

Period:	1	2	3	4
Dates:	16-21.6.61	21-26.6.61	26-3.7.61	3-8.7.61
Diet	172.8	169.7	238.3	165.9
Reject	14.8	10.6	22.5	13.8
Intake	158.0	159.1	215.8	152.1
Urine	131.3	121.9	167.6	135.2
Faeces	15.6	17.6	21.1	13.1
Output	146.9	139.5	188.7	148.3
Balance/5 days	+11.1	+19.6	+27.1(7 days)	+3.8
Balance/1 day	+2.2	+3.9	+3.9	+0.8

Daily urinary output:

Period No.	Date	Potassium (m.Eq.)
1	16-17.6.61.	24.1
	17-18.6.61.	23.5
	18-19.6.61.	29.7
	19-20.6.61.	25.0
	20-21.6.61.	29.0
2	21-22.6.61.	24.2
	22-23.6.61.	21.6
	23-24.6.61.	30.5
	24-25.6.61.	28.4
	25-26.6.61.	17.2
3	26-27.6.61.	20.9
	27-28.6.61.	17.9
	28-29.6.61.	37.5
	29-30.6.61.	23.3
	30-1.7.61.	22.0
	1-2.7.61.	25.7
	2-3.7.61.	20.3
4	3-4.7.61.	27.8
	4-5.7.61.	25.8
	5-6.7.61.	22.9
	6-7.7.61.	26.9
	7-8.7.61.	31.8

DISCUSSION

Reports of the metabolic effects of various preparations of Human Growth Hormone have been adequately reviewed by Raben (1962) and there is abundant evidence of its potent anabolic actions, which have also been studied in hypopituitary dwarfs (Hutchings, Escamilla, Deamer and Li, 1959; Shepard, Nielsen, Johnson and Bernstein, 1960; Shepard, Waxman, Bernstein and Ferrier, 1960).

The work of Shepard and his colleagues is open to criticism in that the first set of balance studies was done on a "free" diet without the use of stool-markers, and in the second set there was no constant dietary intake, and stools were not analysed. When one is seeking small but significant changes in balance one must analyse dietary intake, faeces, and urine. In view of this, a critical assessment must also be made of Ducharme and Grumbach's report (1961) of the ineffectiveness of Human Growth Hormone in "normal" premature infants, as the dietary intake was not analysed in this study.

Apart from differences which may exist in the properties and potency of Human Growth Hormone preparations, there is no agreement as to the mode of selection of hypopituitary dwarfs for therapy, and little attempt has been made to apply standardised tests of endocrine function. Shepard and his colleagues (1960) defined their patient as a "constitutional or primordial dwarf" on the criteria described by McCune (1943), but this definition was incomplete and their attempts to evaluate pituitary function were poor. Again, Lipsett, Bergenstal and Dhyse (1961) described metabolic studies with Human Growth Hormone in "primordial" dwarfs, and their indices of thyroid and adrenal function were based upon serum levels of protein-bound iodine and cortisol. The value of both these tests has been discussed in the first section of this thesis. One must, therefore, exercise caution in the interpretation of balance studies with Human Growth Hormone which are described in the literature in so-called "hypopituitary" dwarfs.

Nitrogen Metabolism.

There was no significant change in the faecal excretion of nitrogen in any patient after the administration of growth hormone in the present series. A fall in urinary nitrogen occurred in patient no. 12 which was most pronounced in the first twenty-four hours after injection, and which lasted for three days. Apart from a marked reduction in urinary nitrogen

during the second twenty-four hours after hormone, there was no sustained effect in no. 24. Patient no. 31 showed no alteration of urinary nitrogen, but no. 33 revealed a definite trend towards nitrogen retention in the second half of the third balance period, and in the first four days of the last period.

There was a sharp fall in blood urea in patients 12 and 24 within twenty-four hours of injection. This was not marked in no. 31, and in no. 33 the change was most pronounced in the second twenty-four hours after growth hormone. This reduction in blood urea is the earliest effect of growth hormone on human nitrogen metabolism which has so far been described. This is most probably a reflection of the anabolic action of the hormone and it has been observed six to twenty-four hours after a single injection (Pearson, Lipsett, Greenberg and Roy, 1957; Raben, 1959).

In Beck's series (1960), which included one hypopituitary dwarf, the fall in blood urea occurred on the second, third and fourth day after injection and persisted for two to three days. Again, in Beck's patients, decreased urinary excretion of nitrogen was usually demonstrable on the second day after injection, and was maximal on the third to the sixth day.

Raben (1962) has already stated that the distribution of this nitrogen retention is not known, but the failure of growth

which occurs in hypophysectomised animals, and the increased rate of somatic growth which follows growth hormone therapy in these animals, are indications of the endocrine control of protein synthesis. Szego and White (1949) showed that the metabolic effects of growth hormone are seen in fasting as well as non-fasting animals, and they showed that alteration in protein metabolism could be achieved with growth hormone, even where gain in body weight did not occur. They postulated that the primary action of growth hormone was on the liver, and this is suggested by an "intense mobilisation" of lipid to that organ.They claimed that growth hormone could thus exert a sparing action on protein catabolism, thereby making available a greater portion of amino acid nitrogen for protein synthesis. They also suggested that this well-documented lipid mobilising effect (Levin and Farber, 1952; Greenbaum and McLean, 1953, Raben and Hollenberg, 1960), would also provide energy for protein synthesis. Further work by Kostyo and Knobil (1959) has shown that increased cellular penetration of amino acids caused by growth hormone favours enhanced protein synthesis. In addition, there is evidence by Korner (1961) that growth hormone increases the synthesis of protein by isolated liver ribosomes from the hypophysectomised rat.

Calcium Metabolism.

A significant reduction in the faecal excretion of calcium was seen in patients 12 and 24. This occurred in the five-day period following injection and was continued throughout the fourth period in patient no. 24, in spite of a reduced intake. This increased intestinal absorption of calcium has been described by others (Panel appointed by the Clinical Endocrinology Committee of the Medical Research Council, 1959; Fraser and Harrison, 1960; Hanna, Harrison, MacIntyre and Fraser, 1960). Patients 31 and 33 showed no change in the faecal output of calcium.

There was no change in urinary calcium in patient no. 12, but no. 24 had a reduced excretion from the fourth to the eighth day after injection. No. 31 showed a gradual reduction in urinary calcium which was most obvious in the second five-day period after hormone administration. A similar trend was illustrated by patient no. 33 from the fourth to the seventh day after injection; thus no patient showed hypercalciuria which was the most striking metabolic feature described by the Medical Research Council Panel (1960). After a 10 mg. dose of Human Growth Hormone given intramuscularly, Fraser and Harrison (1960) found a moderate increase in urinary calcium which lasted about seven days, and after a dose of 30 mg. an equivalent increase in urinary calcium occurred which lasted for fourteen to twenty-one

days. Little difference was found in urinary nitrogen between the two doses, and Fraser and Harrison (1960) believed large doses of Human Growth Hormone stimulated parathyroid activity, and they claimed that good support for this belief was found in the literature. However, Copp (1955) showed that in rats which were treated with growth hormone, decreased phosphate intake caused a marked mobilisation of skeletal minerals so that levels of urinary calcium rose, and considerable demineralisation of the skeleton occurred. If the phosphate intake was normal, then decalcification did not occur, and Copp concluded that the primary effect of growth hormone on bone was on the deposition of protein matrix. Pearson, Soroff, Prudden and Schwartz (1960) reported that after the administration of Human Growth Hormone in adults, the net calcium balance depended on the intake. If the intake exceeded a certain "critical level", retention occurred; if the intake fell below that level, growth hormone increased the loss.

The effects of growth hormone on calcium metabolism remain controversial, but two points are worthy of consideration. First, the effect of growth hormone on the hypopituitary child is probably different from the effect on the adult, and vastly different from the effect on the hypophysectomised adult with malignant disease. Second, the form of calcium in the dietary intake may be an important factor. The Hammersmith Group (Hanna, Harrison, MacIntyre and Fraser, 1960), employ a low calcium diet

with calcium supplements, whereas in The Hospital for Sick Children balance studies are carried out on the child's usual dietary intake.

In the present series, levels of plasma calcium in patient no. 12 showed no constant features. In no. 24, there was a gradual rise from the fourth to the eleventh day after injection. Beck et al (1960) reported no significant change in serum calcium during growth hormone administration.

Phosphorus Metabolism.

There was a significant reduction in the faecal excretion of phosphate in patient no. 24 in the five-day period which followed the administration of growth hormone. This effect was not seen in the other children. Again, in no. 24, there was a marked reduction in urinary phosphate in the same period, which was followed by a tendency to negative balance in the succeeding five days. Patients 12 and 33 showed no change in urinary excretion, but no. 31 revealed a trend to diminished phosphate excretion in the fourth balance period.

Plasma levels of phosphate in patients 12 and 31 fell to their lowest value by the eighth day after injection. Patient no. 24 showed a rise in plasma phosphate two days after growth

hormone, and this rise tended to be sustained throughout the remainder of the balance period. These findings are similar to those of Bergenstal and Lipsett (1960) and Beck et al (1960), who found phosphorus retention in five subjects and a tendency for the serum inorganic phosphate to rise during short-term treatment with growth hormone. Sustained elevation of serum inorganic phosphate and alkaline phosphatase were only seen during long-term therapy. In hypophysectomised rats and in "pituitary dwarfs" serum phosphorus and alkaline phosphatase increased with prolonged use of growth hormone (Raben, 1959).

Patient no. 12 revealed a significant drop in alkaline phosphatase after injection and this was sustained throughout the remainder of the balance. No change was seen in no. 31.

Potassium Metabolism.

Patient no. 24 showed an increased faecal excretion of potassium in the five days following the administration of growth hormone, which was not seen in the other children. In no. 12 there was a sharp reduction in urinary potassium in the first twenty-four hours after injection, and patient no. 24 showed some reduction in the first forty-eight hours. Both children showed a maximal rise in the plasma level of potassium three to four days after injection. The reduction in urinary potassium paralleled that of urinary nitrogen in these children.

Beck et al. (1960) described similar changes in three of their patients in whom the pattern of nitrogen retention was closely parallel to that of potassium retention. They also found that decreased potassium excretion usually occurred during the first twenty-four hours after the administration of growth hormone.

Patient no. 31 showed decreased levels of urinary potassium in the seventh to the eleventh day after growth hormone but no significant change was seen in no. 33. This patient had a moderate degree of impairment of adrenocortical function. Both children showed no significant change in plasma potassium.

Sodium Metabolism.

In patient no. 31 the faecal values for sodium were almost doubled in the first ten days after hormone administration. No change was apparent in the other children. No. 24 showed an increased urinary excretion of sodium within the twenty-four hours immediately after injection, but this was not sustained. Patient no. 31 had a significant reduction in urinary sodium on the second day, thereafter there was a tendency to less positive balance in the succeeding six days. This was followed by a large excretion on the ninth day. Patients 12 and 33 showed no change in urinary excretion of sodium. Both 12 and 24 experienced a

sharp drop in plasma sodium within twenty-four hours of injection, whereas 31 and 33 showed an increase seven days and four days after hormone, respectively.

Beck and his colleagues (1960) described a positive sodium balance in five patients during the administration of growth hormone and in three of these patients the positive sodium balance coincided with an increase in urinary aldosterone. The significance and interpretation of this finding are not clear because two of Beck's patients, who had a marked increase in urinary aldosterone, showed little change in sodium balance. It was suggested by Beck that this increase was in part dependent on the dose of growth hormone given and he also mentioned the possibility that the growth hormone preparation contained some substance which was capable of stimulating aldosterone secretion.

Venning and Lucis (1960) studied the mechanism of action of human, monkey and porcine growth hormone on aldosterone secretion in human and rat adrenals, and they concluded that growth hormone does not directly stimulate aldosterone secretion in vitro. These workers also found that when growth hormone is administered to intact and to hypophysectomised rats, the adrenals of these animals secrete increased amounts of aldosterone. They also found that the plasma of hypophysectomised rats treated with growth hormone appeared to contain a substance which stimulated aldosterone secretion in vitro.

Plasma Alkaline Phosphatase.

Beck et al. (1960) described a rise in serum alkaline phosphatase only on prolonged therapy with Human Growth Hormone, except in the case of a thirteen-year old hypopituitary dwarf who had an elevated serum alkaline phosphatase before treatment, in spite of adequate amounts of Vitamin D. After two injections of growth hormone the levels of alkaline phosphatase dropped, a similar fall occurring in patient no. 12 after one injection. There was a significant drop in alkaline phosphatase which was sustained throughout the balance period. No change was detected in patient no. 31.

Plasma Creatinine, Plasma Chloride and Plasma $T.CO_2$.

No significant changes were seen in these determinations. Patient no. 31 showed a slight rise in creatinine in the first twenty-four hours after injection but this was not continued. Again, the $T.CO_2$ and chloride in no. 31 showed a tendency to rise from the fifth to the eleventh days after hormone. These findings are similar to those of Beck et al. (1960) and Bergenstal and Lipsett (1960).

The findings in these metabolic studies in children with defective pituitary function confirm the potent but variable

metabolic action of Human Growth Hormone which have been described by others (e.g. Beck et al., 1960; Ikkos and Luft, 1960; Hanna, Harrison, MacIntyre and Fraser, 1960). There was, however, no evidence of increased urinary calcium output after the administration of growth hormone and reasons for this observation have been discussed.

SECTION 3

METHODS OF ASSAY OF HUMAN

GROWTH HORMONE

METHODS OF ASSAY OF HUMAN GROWTH HORMONE

There is no chemical means of measuring Human Growth Hormone and most attempts at estimation have employed a bioassay, the best known being that described by Greenspan, Li, Simpson and Evans (1949). This method is based upon the increase in width which occurs in the proximal tibial epiphyseal cartilage in the hypophysectomised rat, after the administration of Growth Hormone. However, the assay is relatively insensitive and demands large amounts of material.

Reports by Collip and Anderson (1934) and by Young (1938) described the production of antisera to Thyrotropic Hormone and Prolactin respectively, and such early demonstrations of the antigenicity of pituitary hormones were rapidly exploited with the isolation of purified pituitary extracts of high specific activity.

In spite of a reported molecular weight of 27,100 (Li,1957), Human Growth Hormone is a poor antigen, but its antigenicity may be enhanced by the use of a mineral oil and lanolin adjuvant (Ramon, Lemetayer and Richou, 1935) or by the use of Freund's complete adjuvant, which is a suspension of mycobacterium butyricum in light mineral oil (Freund, 1951). Such adjuvants have wide applications and their use has facilitated the production of potent antisera to Human Growth Hormone (Read and

Stone, 1958; Hayashida and Li, 1958), and thereby led to the development of methods of immunoassay (Read and Stone, 1958; Read, 1960).

The work to be described was undertaken in an attempt to establish Read's assay which is based upon the haemagglutination technique described by Boyden (1951) and in which the major assumption is made that Growth Hormone in serum is immunologically related to Growth Hormone obtained from pituitary extracts. The assay may be considered in three stages.

In the first stage, sheep red cells are treated with a dilute solution of tannic acid and then they are incubated with a solution of Human Growth Hormone which is assumed to become adsorbed to the red cell surface. When a constant amount of Growth Hormone coated cells is added to increasing dilutions of antiserum, agglutination occurs where specific antibody is present.

In the second stage of the assay, known concentrations of Human Growth Hormone in decreasing amount are added to a suitable dilution of specific rabbit antiserum, and the Growth Hormone conjugated cells are agglutinated in those tubes which contain insufficient Growth Hormone to inactivate the antibody present.

In the final stage, dilutions of human serum are used in

place of Growth Hormone standards, and the dilution which first inhibits haemagglutination is determined. This dilution contains the same amount of Growth Hormone which inhibits haemagglutination in stage two, and thus the concentration of serum Growth Hormone can be calculated.

Production of Antiserum to Human Growth Hormone.

Two male Dutch rabbits, aged three months and weighing 2.8 and 2.9 kilograms respectively, were bled from the right marginal ear vein into sterile, chemically clean glass containers. The first animal received 1 mg. H.G.H. (M.R.C. Raben preparation) in complete Freund's adjuvant (Difco) subcutaneously and intraperitoneally in a volume of 1 ml. at each site. The second rabbit was given 1 mg. thrice-crystallised Ovalbumin (Light) in the same manner and by the same routes. This constituted a positive control for the assay using a "pure" protein antigen.

Injections were given weekly for four weeks, and after a further test-bleeding, 1 mg. H.G.H. was given in a total volume of 2 ml. H.G.H. diluent (Glaxo) by the left marginal ear-vein in the first animal. The second rabbit was given 1 mg. Ovalbumin intravenously in 2 ml. 0.9% w/v sterile saline.

The presence of antibody to Human Growth Hormone was confirmed by the classical ring precipitin technique and a

precipitate was detected at an antiserum dilution of 1:256. Antibody to ovalbumin was detected by the same method in an antiserum dilution of 1:2048. The animals were bled by cardiac puncture on the forty-third day after the first injection, the blood was allowed to stand at 37°C for one hour, the clots were "ringed", and the sera were left to separate overnight at 4°C. They were then distributed in sterile "bijou" containers and stored at -20°C until further use.

Confirmation of the Presence of Antibody to Human Growth Hormone.

The presence of antibody to Human Growth Hormone was confirmed by precipitin ring test, gel-diffusion in agar (Gell, 1955) and gel-diffusion in tubes (Oakley and Fulthorpe, 1953). The presence of univalent antibody in high titre was confirmed by Dr. F.C.Greenwood by the method of diffusion in cellulose acetate (Kohn, 1960). On preliminary titration of the antiserum against tannic-acid treated, Growth Hormone conjugated sheep cells, an end-point was obtained between 1:25,600 and 1:51,200. Sera obtained before injection of the antigen gave no precipitates in the systems described, nor did they cause haemagglutination of Growth Hormone conjugated sheep cells.

Gell diffusion studies with this and subsequent antisera showed no precipitation with ovalbumin (Light) Human Serum Albumin (Lister Institute, Batch no. EPA 133) and porcine adrenocortico-

trophic hormone (Armour, Batch no. EE 1004). The first antiserum gave no precipitate with Human Gamma Globulin (Lister Institute, Batch no. EG 105) but a later preparation formed a well-defined band on diffusion against the same batch of gamma globulin. It was not possible to carry out diffusion studies against preparations of other human pituitary hormones because they were not available at that time. Attempts to demonstrate precipitation by immuno-electrophoresis on cellulose acetate (Kohn, 1960) were unsuccessful.

Method of Assay.

The method followed was that of Read (1960) with certain modifications.

Glassware.

All glass-ware was cleansed in hot "Pyroneg" solution, washed overnight in cold running water, and finally rinsed ten times in ion-free distilled water. All haemagglutination tubes (soda-glass) were carefully inspected before use to ensure (as far as possible) uniformity of the inner bottom surface. Irregularities in the glass were found to cause distortion of the haemagglutination pattern.

Buffer Solutions

The buffers were prepared in accordance with the directions given by Read (1960). In initial experiments, solutions were prepared from stock, but in later experiments all buffers were freshly made up the day before an attempt at assay. All chemicals used were A.R. quality.

Guinea Pig Sera.

Guinea pig blood was obtained by cardiac puncture from old boar guinea pigs. It was allowed to clot, and after one hour at 37°C, the clots were "ringed" and the sera separated overnight at 4°C.

Cells.

All untreated red cells were washed and spun in an M.S.E. "Major" centrifuge at 2,500 r.p.m. Tannic acid treated cells and Growth Hormone conjugated cells were centrifuged at 2,000 r.p.m. The percentage composition of each red cell suspension was determined on a micro-haematocrit (Hawksley).

Results.

Human group O Rhesus positive cells were used initially

because a ready source was available, but no consistent results were obtained in the antiserum titration. Guinea pig cells and formalinised sheep cells (Burroughs Wellcome) were also unsuccessful. Finally, it was decided to use fresh sheep cells (Burroughs Wellcome) and it was possible to titrate antiserum on three successive weeks and obtain good reproduction (a one or two tube difference) on titrations set up in triplicate.

Similar findings were obtained on titration with Growth Hormone standards, but stage three of the assay, using unknown sera, gave inconsistent results. At times an end-point could be read but repetition within two days showed complete haemagglutination in all the tests. On other occasions an end-point would be determined but some or all of the controls would show complete haemagglutination.

On enquiry, it was found that the sheep cells (Burroughs Wellcome) were pooled on the day of collection, and the time from collection to delivery was subject to one or two days variation. Following the example of Ehrlich and Randle (1961), one sheep was used for bleeding. A Southdown ram was kept for this purpose at the country branch of the Hospital for Sick Children, and this was bled as required by external jugular puncture. These cells gave satisfactory results, reproducible to within one or two tubes on three successive occasions at weekly intervals in stages one and two of the assay, but again the assay of human serum was unsatisfactory.

Two technical problems were encountered in the method and these could not be overcome. The first was concerned with the preparation of the tannic acid treated, Growth Hormone conjugated cells. It was not possible to use these cells within thirty minutes of preparation in the third stage of the assay, and there was a possibility that elution of the hormone might have occurred within that time, although attempts to demonstrate hormone in the supernatant by diffusion on cellulose acetate were unsuccessful.

The second problem was met in the preparation of a uniform suspension of "tanned" or conjugated red cells. It was found that satisfactory red cell suspensions could only be made by agitation on a "Vortex" eccentric mixer and this invariably gave rise to red cell breakdown which could be detected as a faint ragged line around a non-haemagglutinated "button" of cells in control tubes, when the mechanical mixer was used.

In view of the difficulties in standardising all the steps in Read's method and because of the disadvantage in having an end-point which is subjectively determined, the procedure was abandoned.

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